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BLOOD TRANSFUSION STUDIES

III. The Relationship of Hemoglobinemia and of the pH of the Urine to Renal Damage Produced by Injection of Hemoglobin Solutions Into Dogs

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HEMOGLOBINURIA occurring after hemolytic transfusion reactions has been a major problem in the use of whole blood transfusions since their inception. In 1667 Jean Baptiste Denys[13] first described the sequelae of a transfusion of incompatible whole blood (sheep blood). It is well known that serious reactions still occur after transfusion in spite of tests for compatibility, etc. There have been a large number of reports of anuria and death from transfusions since 1900. Hesse[50] received answers to questionnaires sent to 1,700 clinics and hospitals all over the world. Two hundred seventeen cases of hemolytic shock following transfusions were reported. Out of 200 cases in which definite statements were made as to the outcome, 105 of the patients died, or a mortality rate of 52 per cent. Other authors[13, 21, 45, 68] have reported small series of cases with comparable mortality rates. Hesse[49] also summarized the information available on the use of universal donors blood in the transfusion of patients of other groups and found twenty deaths occurring in a series of forty-eight reactions. Other isoagglutinins such as the anti-Rh agglutinin were not excluded in this group of cases, so high titers of agglutinins in the donors plasma cannot be incriminated in all of these cases. Hardin[47] compared two series of transfusions in the Army Medical Service. There was no difference in the reaction rates between the 7,299 transfusions of group specific blood and the 9,392 transfusions of universal donor blood. Blackwater fever is the most important cause of death of all patients with hemoglobinuria, for there are a large number of cases each year in tropical areas, and the mortality rate is approximately 25 per cent.[38, 65, 79] Recovery from either condition may occur after virtual anuria or oliguria of as much as a week's duration.[8, 15, 20, 31, 41, 45, 56, 66, 68, 82]

Pathology of the Renal Lesion in Clinical Hemolytic Conditions.—The mechanism of renal damage has been the subject of many pathologic studies in the past. Werner[88] and Barratt and Yorke[8] demonstrated that the kidneys of patients dying from blackwater fever contained granular material in the lumina of the tubules and attributed the urinary suppression to mechanical blockage of the tubules. Ayer and Gauld,[5] Baker and Dodds,[6, 7] Bordley,[13] Daniels and associates,[21] DeGowin and co-workers,[22-24] Goldring and Graef,[45] and Lindau[60]

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have contributed much to the study of clinical cases of hemolytic transfusion reactions and the pathologic study of the renal damage produced. Very little attention has been given to hemoglobinemia. More surprising still, hemoglobinuria itself is not mentioned in some cases and is mentioned in a cursory manner in most reports.

The renal lesions after transfusion reactions have been compared with those in other hemoglobinuric states, namely, blackwater fever,[8, 90] acute hemolytic anemias from drug administration,[37, 83] burns,[81] and crush injuries.[16] March hemoglobinuria,[44] paroxysmal nocturnal hemoglobinuria,[46, 52] and paroxysmal cold hemoglobinuria[64] usually do not cause renal damage. Possible explanations for this will be discussed later. DeGowin and co-workers,[22, 24] Lindau,[60] Bordley,[13] and others[18, 21, 45, 74, 83, 90, 92] agree in all essential details about the pathologic findings. There are degenerative changes in the tubular epithelium, especially of the loops of Henle and distal convoluted tubules, interstitial edema in the corticomedullary portion, granules of hemosiderin in the cytoplasm of the cells of the proximal and distal convoluted tubules, and the appearance of a variable number of deeply pigmented casts in the lumina of the loops of Henle, distal convoluted tubules, and the collecting tubules. The degenerative tubular changes vary from mild vacuolization to necrosis and desquamation of cells. Calcium deposits in the degenerated tubules and definite signs of regeneration of epithelial cells have been found.[45]

The importance of the pigment casts has been a major point of dispute among students of the pathology of transfusion reactions and blackwater fever. Baker and Dodds[6] are of the opinion that the casts cause obstruction of the individual nephron and, hence, cause renal insufficiency. Many other investigators[55, 74, 85, 91, 92] agree with this concept, but there are some who believe that the cast formation is only secondary to the tubular epithelial degeneration and not the cause of renal insufficiency.[5, 25, 45, 60] In their study of nine cases DeGowin and co-workers[24] concluded that several patients had evidence of renal tubular obstruction sufficient to cause uremia but that most patients had evidence of tubular epithelial cell necrosis and interstitial edema as the predominant lesion. Thus, they thought that there are several factors which operate in cases of hemolytic transfusion reactions in human beings. Others[13, 18, 21] have also taken this intermediate position.

The liver is likewise involved in cases of hemolysis from any cause.[45, 60] There is a variable amount of central necrosis of the lobules of the liver. In some cases of blackwater fever and of transfusion reaction this is very severe, with the pathologic appearance of acute atrophy of the liver. The reticulo-endothelial cells of the spleen, liver, and lymph nodes contain a large number of hemosiderin granules.

Experimental Production of Renal Damage by Injection of Hemoglobin Solutions.—Many attempts to reproduce the renal damage in experimental animals have been partially or completely successful. Ponfick[76] in 1875 was the first one to attempt to reproduce the lesions occurring in blackwater fever. He found that the injection of heterologous blood into dogs, cats, and rabbits resulted in hemoglobinuria and the deposition of pigmented casts in the tubules of

the kidney. Levy[59] injected pure hemoglobin solution and both hemologous and heterologous blood cells into rabbits. He observed that the epithelium of the proximal convoluted tubules became swollen but retained its staining quality. Casts appeared in the loops of Henle and the collecting tubules. In some tubules the epithelium was entirely denuded.

Yorke and Nauss[94] injected homologous hemoglobin solution into rabbits and found that unless the rabbits had been taking a dry diet before the injection and unless the hemoglobin solution was concentrated anuria did not occur. The largest dose used by them was 13 Gm. at a single injection. The renal pathology was reported to be like that of blackwater fever resulting in urinary suppression. Barratt and Yorke[8] showed that if hemolyzed blood were centrifuged to remove the stroma of the red cells much larger amounts were then tolerated by rabbits. Sudden death resulted during or immediately after injection when the stroma was not removed. Other authors[6, 12] have had similar experiences. Levy,[59] however, was unable to produce any renal lesions by injecting erythrocyte stroma alone.

Baker and Dodds[6] reported results of three rabbit experiments and gave very incomplete protocols as to dose, pH of urine, and details of pathology. When the injected rabbits were getting a diet with an acid ash, they found that the urine was brown in color and contained much sediment; however, when the rabbits were getting an alkaline ash diet of green vegetables, the urine was red and clear. The acid urine contained methemoglobin and oxyhemoglobin, but the alkaline urine contained only oxyhemoglobin. They concluded that intravascular hemolysis resulting in suppression of urine caused blockage of the renal tubules only when the urine is acid.

DeGowin and co-workers[23, 24] injected hemolyzed dog erythrocytes into a number of dogs under various experimental conditions. Some of the dogs were fed an alkaline ash diet and the others a cooked meat diet with 8.0 Gm. of ammonium chloride daily. The doses of hemolyzed erythrocytes were measured in terms of the volume of packed red blood cells (unwashed) and ranged from 3.0 to 35.0 c.c. per kilogram of body weight with an average of 10 c.c. per kilogram. When the urine was alkaline, four dogs received thirty-two transfusions of more than 10 c.c. of hemolyzed red blood cells per kilogram but did not develop fatal renal insufficiency; however, when the urine was acid, seven out of twelve dogs given comparable doses of hemolyzed blood cells died of uremia, frequently with terminal rigidity and convulsions. Three died after a single transfusion, and four dogs died from uremia after an injection of 9.5 to 10 c.c. of hemolyzed packed red blood cells per kilogram. DeGowin and co-workers also gave small doses of hemoglobin (2 c.c. of packed cells per kilogram body weight) daily for thirteen days to a dog with acid urine and to one with alkaline urine; they found no evidence of renal damage in spite of continuous hemoglobinuria. The kidneys of the dogs dying from uremia had many large casts in the loops of Henle and distal convoluted tubules and degeneration or necrosis of the tubular epithelium in some areas. Several of the dogs with neutral or alkaline urine during injections were sacrificed when in good health. There was no evidence of cast formation or damage to the renal epithelium in these dogs.

Wakeman and associates[85] were able to produce anuria and death in dogs by intraperitoneal injections of large doses of hemoglobin solutions. The pathologic appearance of the kidney was similar to that noted by DeGowin and co-workers. Many others have failed to produce renal damage but have used much smaller doses and have paid little or no attention to this fact. Foy and associates[40] have criticized DeGowin's work stating that extrarenal factors of dehydration, hypochloremia, or acidosis probably accounted for the results obtained in acidified animals. However, DeGowin and co-workers specifically state that their animals were maintained in good condition on the acid diet for months without losing weight and that the carbon-dioxide combining power and plasma chloride levels were within normal limits before injecting hemoglobin solutions. The vomiting occurred only after animals had been injected. Some of these animals developed hypochloremia and alkalosis from vomiting after uremia had set in. In one of Bordley's cases[13] hypochloremia was not a feature in spite of the development of uremia. DeGowin and Baldridge[22] found normal carbon-dioxide combining power and chloride concentrations in their patients at the time of transfusion reactions. In the clinical history of patients with urinary suppression due either to transfusion reaction or to blackwater fever, the oliguria or anuria usually developed before any dehydrating factors such as vomiting could occur. A patient with blackwater fever reported by Wakeman and associates[85] had moderate depletion of chlorides on the day following development of hemoglobinuria. Nausea and vomiting in this patient were severe.

Bing[11] used solutions of oxyhemoglobin and methemoglobin made from crystals of the respective pigments instead of hemolyzed blood cells as used by most other investigators. However, the largest dose of hemoglobin used was 13.0 Gm., or a dose about one-third of the average amount used by DeGowin. Bing made a careful study of tubular and glomerular function. He was able to produce profound renal damage in dogs rendered acidotic by large doses of ammonium chloride following injection of 3.5 to 13.5 Gm. of methemoglobin solutions. Marked tubular damage and only rare pigment casts were observed microscopically in the kidneys of these dogs. Dogs with a normal alkali reserve did not develop any decrease in renal function when 5.5 to 8.1 Gm. of methemoglobin were injected. No decrease in function was observed in either normal or acidotic dogs when oxyhemoglobin solutions were injected. Bing produced an actual acidosis and not just an acid urine.

Aubertin and co-workers[4, 58] were able to produce varying degrees of renal insufficiency in dogs by the injection of varying doses of hemoglobin solutions after preliminary bleeding of the dogs. The largest doses uniformly caused severe renal injury or death from anuria. Those dogs with transient oliguria recovered completely, while those with longer-standing oliguria developed chronic renal insufficiency and death. In the pathologic material they found many pigment casts and were thus led to stress the obstruction of the tubules. Epithelial degeneration occurred also. No attention was given to the reaction of the urine.

The Mode, Site, and Amount of Hemoglobin Excretion by the Kidney.—Many investigators have studied the mode and site of excretion of hemoglobin by the kidney and the renal threshold for hemoglobin in man and experimental animals. Bieter[10] showed that hemoglobin was not excreted by the aglomerular toadfish but was excreted by the catfish and the eel in comparable doses. Doubling the dose of hemoglobin failed to produce hemoglobinuria in the toadfish. Albuminuria could not be produced in the toadfish either. Webster and associates[86] were unable to produce hemoglobinuria in frogs by perfusing the renal portal system with hemoglobin solution, but they were able to produce it by perfusing the aorta of the same frog preparations. Newman and Whipple[70] showed that repeated injections of hemoglobin in doses below the minimal renal threshold for excretion resulted in no deposit of iron pigment in the convoluted tubule cells. Lison[61] injected hemoglobin solution directly into the tubule lumen and found absorption of the hemoglobin granules into the tubule cells. He also ligated the renal artery of frogs and injected hemoglobin solution. No hemoglobin was excreted by the kidney and no granules of pigment appeared in the tubule cells. Yuile[96] agrees that hemoglobin escapes only by way of the glomerulus. The renal thresholds for hemoglobin can be explained on the same basis as thresholds for glucose and other substances, namely, that the tubule cells reabsorb all hemoglobin excreted up to a certain level.

Monke and Yuile[69] determined the simultaneous plasma clearance rates for creatinine and hemoglobin when plasma hemoglobin concentrations were about 250 mg. per cent. Hemoglobin clearance was found to be 3 per cent of that of creatinine. They found renal thresholds in a large series of dogs to vary from 80 to 150 mg. per cent. Yuile and Clark[98] also determined the myohemoglobin clearance rate and found it to be 55 to 60 per cent of that of creatinine. The difference can be explained on the basis of the molecular weight of the two hemoglobins. Myohemoglobin has a molecular weight one-fourth of that of hemoglobin, or approximately 17,000. Monke and Yuile[69] postulate that only 3 per cent of the glomerular pores are electrostatically large enough to allow the escape of the hemoglobin molecule. The uniformity of the process indicates that it is not a matter of glomerular injury.

The threshold for hemoglobin has been determined in human subjects a number of times. Sellards and Minot[80] showed that injection of hemoglobin solution into human subjects could be carried out without untoward reactions. They used as much as 33 c.c. of packed red blood cells hemolyzed with distilled water. Gilligan and co-workers[43] injected 1.3 to 16.4 Gm. of hemoglobin in the form of hemolyzed red blood cells into fifteen human subjects. When the plasma level was increased above 135 mg. per 100 c.c., hemoglobin appeared in the urine. When this level was once exceeded, hemoglobin continued to appear in the urine until the level dropped to 30 to 50 mg. per 100 cubic centimeters. They found that albumin was excreted in addition to hemoglobin, even when there was no pre-existing albuminuria, and continued to be excreted for a short time after hemoglobinuria ceased.

Ottenberg and Fox[73] injected 3.47 to 8.25 Gm. of hemoglobin as hemolyzed red blood cells into twenty subjects. They found that the renal threshold was quite variable and that five of thirteen subjects with plasma levels from 200 to 288 mg. per 100 c.c. did not have hemoglobinuria. When the plasma hemoglobin level did not exceed the threshold, the curve of disappearance of hemoglobin from the plasma was a straight line.

O'Shaughnessy and associates,[72] Duesberg,[27] and Fairley[32-34] also have injected hemoglobin solutions into human subjects. The largest dose injected into any patient was 50 Gm.,[72] and this amount produced some subjective symptoms but no renal injury. Hemoglobinuria lasted thirty hours. Other investigators also have noted rigors and some pain in the muscles and back from injections of hemoglobin. Duesberg[27] showed that the bilirubin concentration of the serum had increased thirty minutes after injection was completed and reached a peak a variable time later depending on the dose of hemoglobin and the patient's condition. Ottenberg and Fox[73] confirmed this observation.

The amount of hemoglobin excreted by the kidney after intravenous injection of a solution depends on the dose. Drabkin and associates' statement[26] that 9.5 per cent of the injected dose is excreted has been quoted widely, but it has been clearly demonstrated that the percentage varies with the dose injected unless oliguria or anuria are produced and that it may be as great as 33 per cent of the initial dose.[43, 72, 96] Likewise the duration of hemoglobinuria varies directly with the dose injected but also depends somewhat on the general condition of the patient or animal.

Reid[77] placed the kidney of a number of dogs in collodion plethysmographs and determined the effect of the injection of distilled water on the renal volume. There was a transient marked decrease in the volume of the kidney after the injection of 0.5 to 1.5 c.c. of water for each kilogram of body weight. The shrinkage lasted one or two minutes. He also injected laked dog blood made isotonic with sodium chloride. Five cubic centimeters of laked dog red blood cells caused the most marked decrease in renal volume, lasting up to twelve minutes. Anesthesia did not abolish the decrease in volume. A denervated kidney responded the same as the normal kidney. Mason and Mann[67] made direct observations on the frog kidney following the intravenous administration of a solution of pure hemoglobin and concluded that the decrease in volume of the kidney resulted from generalized contraction of the renal arterioles and arteries.

Hesse and Filatov[48] found that the injection of homologous hemoglobin solutions caused decrease in the renal volume lasting from several to twenty-five minutes, depending on the dose of hemoglobin. Heterologous blood caused a similar shrinkage of the kidney. Initially they found that complete denervation of the kidney prevented the shrinkage of the observed kidney. However, in later experiments by a group of investigators[89] from the same laboratory, it was clear that decerebration, transection of the cord, cutting the splanchnic nerves, and denervation of the kidney did not prevent the shrinkage; therefore, they concluded that the hemoglobin itself caused spasm of the renal vessels.

Hesse and Filatov[48] showed that transfusion of compatible blood promptly relieved the spasm of the renal vessels, that is, the volume and pulsations of the kidney returned quickly to normal. On the basis of this evidence they recommend the use of compatible blood transfusion as a treatment of hemolytic transfusion reaction. They believed that all the manifestations of hemolytic transfusion reactions could be explained on the basis of spasm of renal vessels, but later reports by members of the same group, Iljin and Mincev,[53, 54] conclude that some factor in addition to spasm accounts for renal damage.

Amberson and co-workers[2] were able to replace completely the blood of cats, dogs, and rabbits with a Ringer-Locke solution containing 12 to 14 Gm. of beef hemoglobin per 100 cubic centimeters. The animals were able to walk around the laboratory but they did not eat or drink and anuria developed. Some lived as long as thirty-six hours.

De Navasquez[25] injected hemoglobin solutions into rabbits and determined the iron content of the kidneys. He found that the animals excreting acid urine retained less iron and for a shorter time than those excreting alkaline urine. On the basis of this evidence he concluded that the rabbit was able to excrete hemoglobin better when the urine was acid. The dose of hemoglobin was relatively small, less than 1.0 Gm. per kilogram. Foy and associates[40] cite evidence that the mammalian kidney is better able to excrete solids when the urine is acid than when the urine is alkaline. This argument, however, does not take into account the matter of solubility of hemoglobin at various pH levels.

Maegraith and Havard[65] summarized mortality statistics of blackwater fever before the use of alkalies and after their use. In their summary of the series of cases reported by three different authors the mortality rate ranged from 19 to 25 per cent before the use of alkalies. In five different reports, but of a smaller number of patients receiving alkalies, the mortality rate ranged from 20 to 37 per cent. Maegraith and Havard concluded that alkali therapy has not benefited a group though it may have helped individual patients. They also pointed out the fact that patients with oliguria or anuria often continue to have an acid urine in spite of continuing alkali therapy. Anuria developed in some cases where the urine was alkaline immediately before the hemoglobinuria and continued to be alkaline after oliguria commenced. Foy and Kondi[39] likewise have observed that patients with alkaline urine develop anuria.

De Navasquez[25] acidified the urine of a patient with paroxysmal hemoglobinuria due to cold and then induced an attack by immersion of the arm in ice water. Hemoglobinuria lasted six hours, the highest plasma hemoglobin value was 688 mg. per 100 c.c., and an estimated 13.0 Gm. of hemoglobin were liberated. No renal insufficiency developed, and De Navasquez cites this as evidence that an acid urine is not a factor in renal damage resulting from hemoglobinemia. This, however, represents but a small amount of hemolysis, and it is probably incorrect to draw conclusions one way or the other from De Navasquez' experiment.

There are several considerations which are overlooked in most discussions of hemoglobinemic states, and it would seem that the most important of these is

the actual amount of hemoglobinemia present at the height of the reaction prior to the onset of oliguria or anuria. It is insufficient to determine the level of plasma hemoglobin a day or two after the onset of hemolysis, for it has been amply demonstrated that hemoglobin is rapidly removed from the blood stream in man and in animals.[33, 34, 43, 72, 73] Foy and Kondi[38] determined hemoglobin concentration of their patients with blackwater fever twelve to twenty-four hours after onset. Though they make a statement that the amount of hemolysis seems to be unimportant, their protocols indicate that the patients who died from anuria had the highest levels of plasma hemoglobin. Fairley and Bromfield[31] found that the highest hemoglobin concentrations occurred in their three fatal cases (3.6, 4.6, and 5.14 per cent of normal hemoglobin concentration in these cases).

The plasma level of hemoglobin was determined in four cases of hemolytic anemia due to sulfonamides by Fox and Ottenberg.[37] Twelve to forty-eight hours after the onset of hemolysis the total hemoglobin pigment levels ranged from 0.4 to 1.7 Gm. per 100 cubic centimeters. Two patients died from anemia and renal insufficiency. At autopsy on one patient dying three days after onset, the kidneys showed a large number of hemoglobin casts chiefly in the loops of Henle and distal tubules. Foy and associates[41] studied a patient dying from uremia as a result of an acute hemolytic anemia due to benzyl sulfanilamide. Quantitative determinations of the urine and plasma pigments were carried out, but no mention is made of the hemoglobin level in the plasma until the fifth day after jaundice was noted. At that time there were 291 mg. of methemalbumin (the pigment described by Fairley[34]) and 90 mg. of oxyhemoglobin per 100 c.c. but no methemoglobin.

The plasma hemoglobin levels in cases of paroxysmal nocturnal hemoglobinuria (Marchiafava-Micheli type) studied by Ham[46] and Hoffman and Kracke[52] ranged as high as 279 and 288 mg. hemoglobin per 100 c.c. of plasma Ham reports one death from renal insufficiency but this patient had an acute pyelonephritis. Altschule and Gilligan[3] report a case of acute massive hemoglobinuria with jaundice and anemia. The nonprotein nitrogen rose to 90 mg. per 100 c.c. of blood during the course of the hemoglobinuria but returned to normal afterward. The plasma hemoglobin level was 500 mg. per 100 c.c. twenty-four hours after the onset, but it may well have been higher at some time earlier. Hemoglobinemia continued for eighteen days.

Recently, Shen and co-workers[81] studied eleven patients with hemoglobinuria out of forty with extensive second and third degree burns. Plasma or serum hemoglobin levels ranged from 65 to 215 mg. per 100 c.c. at an unspecified time after the burn. In four out of five patients with hemoglobinuria who lived five days or longer, azotemia of moderate severity occurred in spite of re-establishment of adequate urine flow with fixed low specific gravity of the urine. The kidneys of six patients were studied histologically and were consistent with hemoglobinuria. Olson and Necheles[71] studied burns experimentally and found that renal insufficiency was most likely to occur when the plasma hemoglobin level was highest. They found levels as high as 2,000 mg. per 100 cubic centimeters.

EXPERIMENTAL METHODS

Animal Experiments (Explantation of One Kidney).—Explantation of the kidney has been carried out many times in the past. The first mention of this procedure is made by Allen in 1925.[1] Loesch[62, 63] explanted kidneys for the purpose of study of experimental hypertension. The renal pedicle was clamped intermittently and hypertension was produced. Biopsies were obtained by subjecting the dog to a surgical procedure. Entz and Huggins[29] explanted the kidney extracutaneously and were able to protect it sufficiently to prevent ulceration until a thick leathery capsule developed. Total kidney function as measured by the phenolsulfonphthalein test was unaffected by the procedure. Rhoads[78] carried out a somewhat more elaborate procedure in that the renal vein was also anchored subcutaneously so that repeated samples of renal vein blood were obtainable. Explantation of one kidney caused no change in the urea clearance. Removal of the other kidney caused no greater fall in urea clearance than if the remaining kidney had been in normal position. Gabriele[42] showed that the renal blood flow remained the same if the kidney were displaced outward and downward. However, if the renal pedicle were moved upward to form an acute angle with the great vessels, renal blood flow in that kidney decreased. Autopsies in the dogs used in the present experiments revealed an angle of 90 degrees or more with the great vessels and no kinking or stretching were found. Explantation of a kidney does not alter its blood flow appreciably.

Mongrel dogs weighing from 6.25 to 13.6 kilograms were chosen as the experimental animals. Each dog was anesthetized with sodium pentobarbital given intravenously. An oblique incision was made along the right costal margin beginning about 3 cm. from the midline dorsally. The muscle layers immediately caudal to the costal margin were split along the direction of their fibers. The peritoneum was incised and the kidney freed from its peritoneum and perinephric fat by blunt dissection. Care was taken not to dissect around the renal pedicle for the kidney could be mobilized adequately without interfering with the pedicle. The kidney was delivered through the opening in the muscle layers and rotated through an arc of about 75 degrees so that the upper pole was directed dorsally and slightly cephalad. The peritoneum and muscle layers were closed loosely around the pedicle using silk or linen suture material. Finally the skin was sutured using sulfathiazole powder along the incision. No dressings were applied. The skin sutures were removed on the sixth or seventh days. In several dogs there was a minor separation of the skin margins which healed satisfactorily in a short while. One dog suffered a wide separation of the skin margins on the fifth day directly exposing the kidney. The kidney became ulcerated and finally it was necessary to sacrifice the dog without using it for an experiment. This was the only failure out of eleven dogs operated upon. The postoperative tissue reaction usually subsided in two or three weeks. The kidney was mobile to a certain extent but often the skin was attached to it.

Biopsy Technique.—When the reaction to operation had completely subsided the area over the explanted kidney was shaved and prepared with Novak's antiseptic. One per cent procaine hydrochloride solution was used as a local anesthetic. A Silverman needle* was used to obtain biopsies of the kidney. Tripoli and Fader[84] and Hoffbauer[51] have used this instrument for biopsy of the liver. The author is indebted to Dr. Hoffbauer for helpful suggestions regarding the use of the needle. The poles of the kidney were used for biopsy sites for the most part. The needle was directed in such a way as to avoid the hilus region. The core of kidney tissue removed was placed in 10 per cent formalin in physiologic saline solution. As soon as the needle was removed the kidney was compressed between two fingers for a period of three to five minutes; it was possible to prevent hematoma formation at the site of biopsy by this compression. About 80 per cent of attempted biopsies were successful in obtaining satisfactory tissue for sectioning. In earlier experiments biopsies were obtained prior to any injection, but in later experiments biopsies were obtained only after injection. Sixty satisfactory biopsies were made and as many as ten from a single kidney were obtained.

*Vim No. 14 made by the MacGregor Instrument Co., Needham, Mass.

The specimens were mounted in paraffin and stained with hematoxylin eosin stain. Some of the autopsy specimens were also stained for free iron using potassium ferricyanide and hydrochloric acid.

Biopsies were not entirely innocuous in every instance. One animal (Dog 6) died as a result of abscesses in the lower pole of the kidney at the site of a biopsy, and another (Dog 11) had a small abscess at one pole of the kidney. Dog 9 had a hematoma in the lower pole of the kidney as a result of the operation or of the control biopsy, most likely the former. At autopsy most of the kidneys were pitted at the site of some biopsy but only very small areas were involved.

Preparation of Hemoglobin and Hemolyzed Red Blood Cell Solutions.—Blood was obtained from dogs which were to be sacrificed for various reasons but were not ill. The blood was collected in sterile containers with sodium citrate as an anticoagulant. The red blood cells were allowed to sediment for four or five days, the plasma was removed by aspiration, and the remaining cells were washed three times with sterile sodium chloride solution. (The cells were not washed for Experiments 2 through 4). The washed cells then were stored in a deep freezing unit (the cells were laked immediately for experiment 7b). The night before contemplated use two volumes of sterile distilled water were added to the cells. After several hours an amount of sterile hypertonic sodium chloride solution was added to make the solution isotonic. The laked cells were then centrifuged for one hour and the supernatant solution was removed by aspiration. The process of laking the cells and making the solution was carried out in the twelve-hour period before the use of the solution which was stored in a refrigerator all the time except during centrifugation. Spectroscopic examination of each solution was carried out immediately before its administration, and oxyhemoglobin alone was observed in all of the solutions prepared by laking the blood cells as previously described. Cultures were obtained on four occasions and no bacterial contamination was found at any time. Sterile precautions were taken in making all of the solutions. The hemolyzed erythrocyte solutions were used in Dogs 2 to 9.

The method of Bing[11] was used to prepare crystalline dog hemoglobin for use in three experiments for Dogs 10 and 11. The blood was collected and treated as described to obtain red blood cells. One-fifth volume of toluene was added to each bottle of washed cells; the bottles were vigorously shaken for five minutes and then allowed to stay in the refrigerator for twenty-four hours. The mixture was then centrifuged and the toluene layer and a creamy layer, presumably the stroma and lipoid layer, were decanted. The crystalline deposit was washed three times with cold sterile distilled water and centrifuged each time. Finally the crystalline deposit was dissolved in sterile saline solution. The crystals were found to be relatively insoluble unless some sterile sodium bicarbonate solution was added. (The amounts will be specified in individual experiments.) Before injection in each instance the solution was centrifuged and the clear supernatant solution used for intravenous injection. Cultures of this solution were found to be sterile in each of two instances in which cultures were obtained. As will be seen, much larger amounts of hemoglobin were used than were used by Bing. Methemoglobin was not detected by spectroscopic examination in the first two solutions used but was detected in the solution used in Experiment 10b and in one solution which was discarded.

Injection Experiments.—Each dog was given either ammonium chloride or sodium bicarbonate by mouth or stomach tube before the injection. At first 10 Gm. of either substance in 100 c.c. of water were introduced by stomach tube. The dogs tolerated the sodium bicarbonate quite well and this method was entirely satisfactory in producing alkaline urine each time it was used. The ammonium chloride was not tolerated well. In most instances it caused vomiting and several dogs exhibited convulsive disturbances immediately after intubation. On these occasions experiments had to be postponed and the hemoglobin solutions discarded. Enteric-coated ammonium chloride tablets given orally in doses of 3 Gm. at approximate intervals of eighteen hours, twelve hours, and one hour before the experiment proved to be very satisfactory in producing an acid urine and were well tolerated. It is difficult to understand how as much as 120 Gm. of ammonium chloride could have been given to dogs in forty-eight hours as described by Bing[11] without causing severe disturbances.

The dogs were anesthetized with sodium pentobarbital given intravenously and catheterized leaving the catheter in place for eight to ten hours after completion of the injection of hemoglobin or hemolyzed blood solution. No. 8 French ureteral catheters were used for male dogs. Blood was obtained for hematocrit determination after which the solution was injected at a rate of about 20 c.c. per minute into a forepaw vein. Subsequently, blood specimens for plasma examination and hematocrit were obtained by venepuncture or femoral artery puncture immediately after completion of injection and at intervals during the first eight hours. Isotonic sodium citrate was used as an anticoagulant in the first four experiments, but a correction for the volume of citrate solution is necessary since an appreciable dilution occurs when one volume of solution to nine volumes of blood are used. For the rest of the experiments one drop of 25 per cent sodium citrate solution per 5 c.c. of blood was used and no correction for dilution was made. Artificial hemolysis is not induced by the 25 per cent sodium citrate when the blood is otherwise handled with care.

Urine was collected at variable intervals depending on the rate of urine flow. An attempt was made to collect separately urine of grossly comparable concentration. After removal of the catheter all the urine was collected by the use of metabolism cages. Freshly voided specimens were collected when possible during the first few days after the injection.

Chemical Procedures and Other Methods.—The concentration of hemoglobin in the solution, the plasma, and the urine was determined by the pyridine hemochromogen method described previously.[35] The urine hemoglobin concentrations were determined directly, that is, without extraction with ether, for the concentrations were great. It was found that no error resulted by using the method described for plasma. Both urine and plasma had such great concentrations of hemoglobin that it was necessary to use 0.02 to 0.10 c.c. samples initially and from 0.1 to 0.5 c.c. later. It is necessary to use the reagent grade of pyridine in order to get uniform results. The method is as follows: The citrated blood is centrifuged as soon as it is obtained and the plasma is withdrawn. Plasma samples (0.02 to 0.05 c.c. or more up to 0.5 c.c.) are placed in Evelyn colorimeter tubes. The plasma is diluted with dilute ammonium hydroxide to 7.5 c.c. and finally to 10 c.c. with 0.5 c.c. of pyridine and 2.0 c.c. of freshly prepared 0.2 per cent sodium hydrosulfite. A duplicate tube for center setting determinations is set up in the same manner but with 1.0 c.c. less ammonium hydroxide, thus allowing for 1.0 c.c. of 3 per cent hydrogen peroxide. Readings are obtained in five minutes. The following formula is used:

$$\text{Concentration} = \frac{1000 \times L}{K \times V} \text{ mg. hemoglobin per 100 c.c. plasma}$$

L - (2 - log G) = "L" value
G - galvanometer readings
V = c.c. of plasma used
K = 1.843.

Hematocrit determinations were made using dry mixed ammonium and potassium oxalate as anticoagulant and the Wintrobe hematocrit tube. Unfortunately, several control specimens clotted so that some of the experiments had to be excluded from certain calculations involving hemodilution, etc. Blood specimens were usually obtained at twenty-four hour intervals for the duration of hemoglobinemia and at variable intervals for the determination of the blood urea nitrogen, depending on the circumstances of each experiment. The blood urea nitrogen was determined by the direct nesslerization of a Folin-Wu filtrate of blood incubated with urease.[75] The method has been adapted for use with the Evelyn colorimeter.

Microscopic examination of urine sediment was carried out at intervals as seen in the tables of each experiment. Only fresh urine specimens were examined.

Spectroscopic examinations were carried out using a Zeiss hand spectroscope. Methemoglobin concentrations in Experiment 10b were carried out by the method of Evelyn and Malloy.[30]

The pH of the urine was determined by the use of a Coleman No. 3 electrometer. When the reaction is recorded as alkaline or acid, alkacid ribbon* was used. The pH was determined in two hours or less after collection directly from the dog.

*Fisher Scientific Co., Pittsburgh, Pa.

EXPERIMENTAL OBSERVATIONS

Nineteen experiments on ten dogs were done. Serial biopsies were obtained in eleven experiments and were satisfactory for pathologic examination. The earliest biopsies were obtained four to eight hours after injection of hemoglobin solutions in Experiments 3, 4, 5a, 6, 7a, 8, 9, and 10b and twenty-four hours afterward in Experiments 7b, 10a, and 11.

Data regarding the volume, quantity of hemoglobin, plasma hemoglobin levels, hemodilution as measured by hematocrit changes, duration and extent of hemoglobinuria, pH of the urine during the first twenty-four hours, blood urea nitrogen levels, presence of casts are recorded in Tables I and II. The data regarding the first four experiments are not included because of the relatively small quantities of hemoglobin injected, but some of these experiments are represented on the graphs listed as Experiment 2a to 2d. Two dogs died shortly after the injection was completed (Experiments 2g and 5b).

Dogs 5a, 6, 7a, 7b, and 10a recovered promptly from the effect of the infusions and anesthesia. Dogs 5a, 6, and 7a had acid urine and Dogs 7b and 10a had alkaline urine. Dog 6 developed an infection (proved to be renal abscesses at autopsy) and became progressively more ill after the fourth day. None of the above dogs developed renal insufficiency. Dogs in Experiments 3, 4, 8, 9, 10b, and 11 developed renal insufficiency at least temporarily or died from uremia. Dogs 3, 4, 8, and 10b had alkaline urine and Dogs 9 and 11 had acid urine. Dog 3 was seriously ill for about three weeks; then it recovered partially but contracted a respiratory infection and died from pneumonia on the forty-sixth day. Dog 8 was ill from the time of injection and contracted a respiratory infection on the fourth day but responded to sulfadiazine therapy and recovered. Dog 4 was ill for three or four days but recovered completely. Dogs 9 and 11 became progressively more ill and had twitchings and convulsions terminally, vomited frequently, and died from uremia. Dog 10b died approximately thirty-six hours after injection; the immediate cause of death was not determined but there was extensive renal damage at autopsy.

One animal (Dog 5b) died three hours after the injection, either from shock or from cardiac irregularity which was noted just before death. There was a moderate amount of free peritoneal fluid which contained 2.5 Gm. of hemoglobin per 100 cubic centimeters. In the other experiments the spleen and liver often were engorged at the time of injection and the veins were greatly distended. In Experiment 2g the dog died about eight hours after completion of the injection. The spleen was large and the peritoneal cavity contained hemoglobin-stained fluid. All the organs had hemorrhagic areas throughout their substance. The lumen of the intestines contained a considerable quantity of blood.

Unfortunately blood pressure determinations were not obtained. From observation of the pulse volume there probably was some lowering of the blood pressure in the first eight-hour period. It is significant that at no time did anuria occur; in other words, the blood pressure did not decrease enough to cause complete failure of excretion of urine.

TABLE I. SUMMARY OF DATA REGARDING DOSE OF HEMOGLOBIN, PLASMA LEVELS OF HEMOGLOBIN, HEMODILUTION, AND HEMOGLOBIN EXCRETION

DOG	WEIGHT (KG.)	HEMOGLOBIN SOLUTION			PLASMA HEMOGLOBIN (GM./100 C.C.) IN HOURS AFTER INJECTION OF HEMOGLOBIN SOLUTION								HEMODILUTION† (%)	EXCRETION (%)	HOURS OF HEMOGLOBINURIA	REACTION OF URINE
		VOLUME (C.C.)	AMOUNT (GM.)	(GM./KG.)	0	½	1	2	4	*	*	*				
2e	13.5	850	32.1	2.38	2.60	2.39	1.96	1.74	1.49	.82(10)	.16(31)		–	28	31	Alkaline
2f	13.5	850	40.0	2.96	3.43		3.29	2.88		1.94(8)	.24(28)		152	35	32	Acid
3	12.7	900	68.8	5.42	4.70	4.18	4.15			2.41(7)	1.30(24)	.32(72)	148	18	72+	Alkaline
4	12.7	700	54.0	4.25	3.75		2.91	2.34		1.37(8)	1.10(24)	.13(72)	180	36	72	Alkaline
5a	8.18	575	36.9	4.50	3.18	2.96	2.74			1.24(10)	.73(27)	.33(48)	170	35	48	Acid
6	6.25	525	30.2	4.80	3.46		2.80		2.02	1.77(8)	.51(24)	.01(72)	156	38	48+	Acid
7a	7.9	590	41.0	5.20	3.12	2.94	2.66		2.40	1.57(8)	.45(24)	.16(48)	137	38	48	Acid
7b	11.3	950	52.0	4.60	3.35	2.89	2.81		2.09	1.81(8)	.63(24)	.09(72)	–	30	48+	Alkaline
8	7.95	700	45.5	5.74	3.32		2.94			2.18(8)	1.41(27)	.34(48)	143	31	72	Alkaline
9	9.1	725	51.8	5.70	4.72			4.12	3.62	3.00(8)	2.45(26)	.47(72)	–	27	72+	Acid
10a‡	6.45	950	39.8	6.15	3.38		3.04			2.48(8)	.67(24)	.10(72)	160	42	72	Alkaline
10b‡	8.1	450	48.4	5.95	5.63	5.35		4.89 (3)		3.83(8)	1.98(26)		192	24	36	Alkaline
11‡	8.18	975	43.8	5.36	3.78	3.55	3.46			2.06(8)	1.09(26)	.21(72)	176	37	Died 72	Acid

*The time in hours is indicated by the number in parentheses.

†Hemodilution is an expression of the ratio of initial hematocrit to the lowest hematocrit after injection.

‡Solutions of crystalline hemoglobin were used in these three experiments.

TABLE II. SUMMARY OF DATA REGARDING BLOOD UREA NITROGENS, URINE pH, AND URINALYSIS

DOG	DOSE OF HEMOGLOBIN (GM./KG.)	MEDICATION IN GRAMS		BLOOD UREA NITROGEN (MG./100 C.C.)					URINE VOLUME							
		$NaHCO_3$	NH_4Cl	TWO DAYS	THREE DAYS	FOUR DAYS	SEVEN DAYS	*	RANGE OF pH FIRST DAY	CASTS	TIME OF APPEARANCE OF METHEMOGLOBIN (HR.)	FIRST DAY (C.C.)	RANGE OF VOLUME FIRST WEEK†	EXCRETION (% OF TOTAL DOSE)	DURATION OF HEMOGLOBINURIA	ALBUMIN SEVENTH DAY
2e	2.38			11					8.1 to 8.2	0		766		27.6	31	
2f	2.9			15					5.9 to 6.5	+	2	1,352		35	32	
3	5.42	10		72	-	92	85	53(46)	7.6 to 8.5	++	10	901	975 to 500	17.5	72+	+
4	4.25	10		40		27	22	18(16)	7.6 to 8.35	+	9	1,040	1,050 to 100	36	72	+
5a	4.5		10	12		11			6.7 to 7.1	+	3	404	200 to 700	35	48	0
6	4.8		7.5	22		53	11		6.0 to 6.7	++	2	450	150 to 1,100	38	48+	0
7a	5.2		9.0		15		17		5.8 to 6.8	++	2	1,283	150 to 1,325	37.8	48	0
7b	4.6	10		6	9	18			7.5 to 8.1	+	5	1,413	200 to 700	29.8	48+	0
8	5.74	11		39	46		31	32(16) 9(26)	7.7 to 8.4	++	8	785	400 to 1,000	31	72	Trace
9	5.7		12	120	200	300	390		5.5 to 6.6	++	1	633	150 to 872	27	72+	+
10a‡	6.15	10		17		13			8.1 to 8.4	Occasional	10	1,090	250 to 440	42.5	72	0
10b‡	5.95	5		56(1)					7.8 to 8.4	+	1	715	-	24	36 death	
11‡	5.36		12.5	49		155	190	278(9)	5.5 to 7.0	++	1	755	350 to 1,100	37	72	Trace

*The time in days is indicated in the parentheses.

†The volumes may not be accurate for the dogs with renal insufficiency after the third or fourth day, since these dogs vomited and the vomitus would be collected just as voided urine would be.

‡Solutions of crystalline hemoglobin were used in these experiments.

An attempt was made to determine the size of the kidney by palpation during the immediate postinjection period. In no instance did the kidney seem to shrink in size, but in several dogs it seemed to enlarge slightly. For several hours following injection the consistency was uniformly firmer than before. External measurements by means of a ruler confirmed the impression that shrinkage did not occur. Of course, there may have been some change in volume which could have been determined only by plethysmography.

From the data in Table II it is evident that methemoglobin appeared in the urine much earlier when the urine was acid than when it was alkaline. In all except Experiment 10b the hemoglobin solution contained no methemoglobin or less than is detectable by spectroscopic examination. The solution used in 10b contained 13 per cent methemoglobin and the rest was oxyhemoglobin.

The results of the renal biopsies can be summarized for Experiments 5a and 5b, 6, 7a and 7b and 10a by stating that little or no renal damage could be detected at any time after injection. Rarely casts were found in some of the tubules during the period of hemoglobinuria and were fairly numerous in Experiment 7a. (Sections are normal otherwise so they will not be illustrated.) The convoluted tubule cells contained many granules of pigment which had the staining qualities of hemosiderin. A few of these granules were found in the kidney as long as 112 days after injection (Dog 4). Hemosiderin granules were numerous in the reticulo-endothelial cells of the spleen and liver, too. The explanted kidney of Dog 6 was partially destroyed by multiple abscesses, but the rest of the kidney and the normally placed kidney were entirely normal at autopsy. The same fundamental structural changes were present in the normally placed kidney as in the explanted one at autopsy in every dog, except when an abscess (Dog 6 and 11) or hematoma (Dog 9) were found in the explanted kidney.

Casts appeared in the urine in abundance in practically every experiment. The casts were brown and granular in appearance. Although quantitative determinations were not made, it was apparent that the number of casts was greater in some experiments when the urine was acid than in any when it was alkaline. The casts were not identified chemically but very likely were derived from hemoglobin because of their brown-red color and their occurrence immediately after hemoglobinuria started. The casts in the tubules of the kidney did not stain with ferricyanide so they did not contain any free iron. Many of the casts in the tubules had a crystalline structure. The casts stained uniformly with the eosin stain and had a color similar to red blood cells stained with eosin.

The pathologic findings in dogs with renal damage were more interesting. The urine was alkaline during the first twenty-four hours in Experiments 3, 4, 8, and 10b and acid in Experiments 9 and 11. An attempt will be made to illustrate early damage, its progress, and finally partial or complete healing. Space does not permit inclusion of description of all of the sections obtained; therefore, only the most characteristic findings will be described.

Four hours after the injection in Dog 3 there was slight vacuolization of the epithelial cells in the proximal and distal convoluted tubules and there were

Fig. 1.

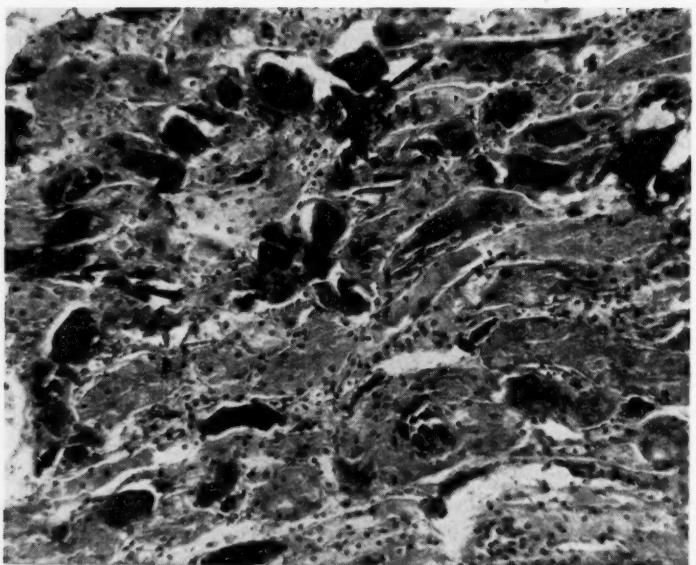


Fig. 2.

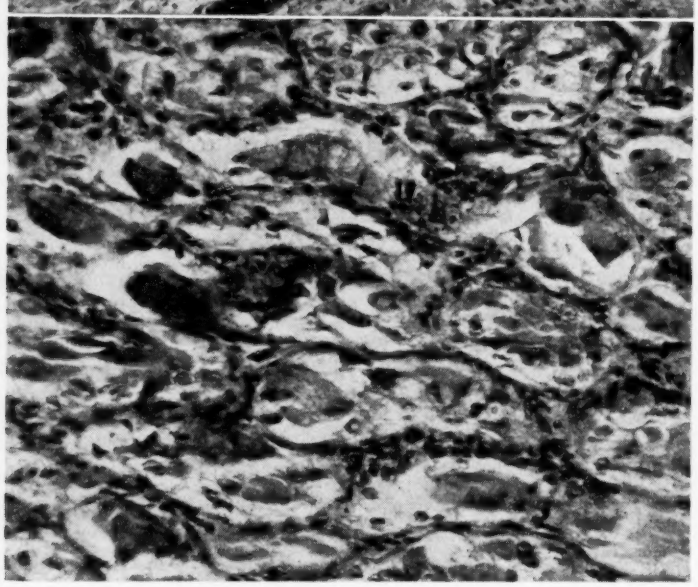


Fig. 1.—Kidney, Dog 3; alkaline urine; biopsy obtained one day after injection of hemoglobin solution. Numerous casts are evident and there are early degenerative changes in the tubular epithelium (×150).

Fig. 2.—Kidney, Dog 3; biopsy obtained five days after injection of hemoglobin solution. There are very extensive degenerative changes in the tubule cells. Other portions of the same section have many hemoglobin casts in the tubules (×300).

Fig. 3.

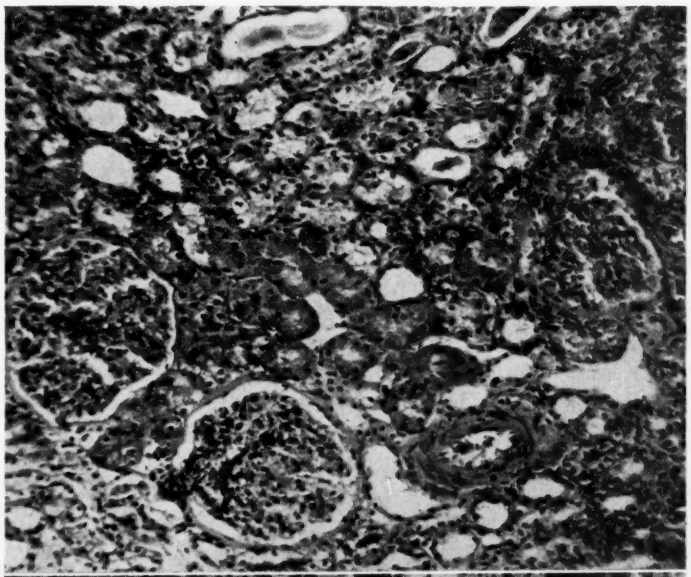


Fig. 4.

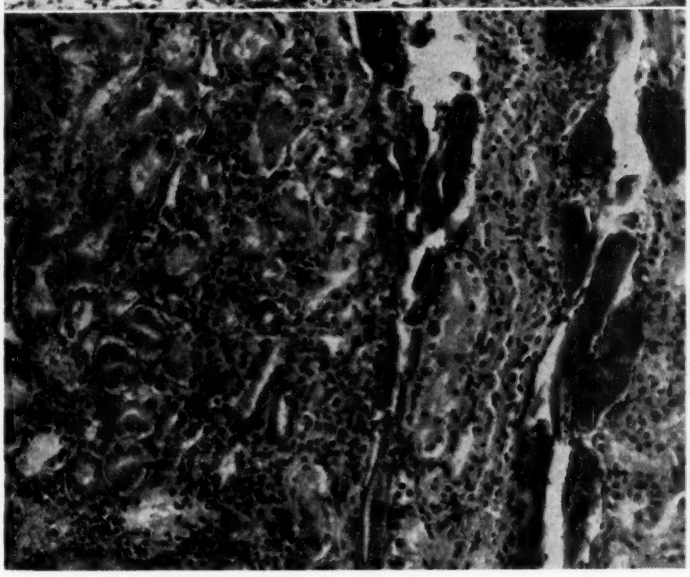


Fig. 3.—Kidney, Dog 3; biopsy obtained seventeen days after injection of hemoglobin solution. The epithelium of the convoluted tubules shows many evidences of healing as well as persisting degenerative changes in other areas (×150).

Fig. 4.—Kidney, Dog 3; autopsy forty-six days after injection of hemoglobin solution. There are narrow wedges of normal cortex interposed between areas of tubular atrophy. The convoluted tubule cells in the atrophic areas have many hemosiderin granules (when stained with a ferricyanide stain). The lumina of the atrophic tubules are narrow but patent. The photomicrograph depicts an area of healed cortex adjacent to one atrophy with large hemoglobin casts (×150).

many hemoglobin casts in the loops of Henle and of the distal convoluted tubules. The cytoplasm of the cells of the proximal convoluted tubules had red staining globules. Twenty-four hours after the injection the lumina of the majority of loops of Henle and Distal convoluted tubules were full of hemoglobin casts (Fig. 1). There was swelling of the cells of some proximal convoluted tubules and the nuclei were pyknotic. At forty-eight hours the tubular epithelium showed further degeneration. At five days extreme vacuolization of the cells and some disorganization of the tubules in both the cortex and corticomedullary portion had appeared (Fig. 2). The number of casts in the corticomedullary portion (not shown in the photomicrograph) was as numerous as on the first day. At ten days there was evidence of beginning regeneration of tubule cells. At seventeen days most of the casts were in the corticomedullary portion. In the cortical portion as shown in Fig. 3, some of the tubules were lined by flat or low cuboidal cells and apparently were undergoing atrophy, but others were regenerating and had nearly normal appearance.

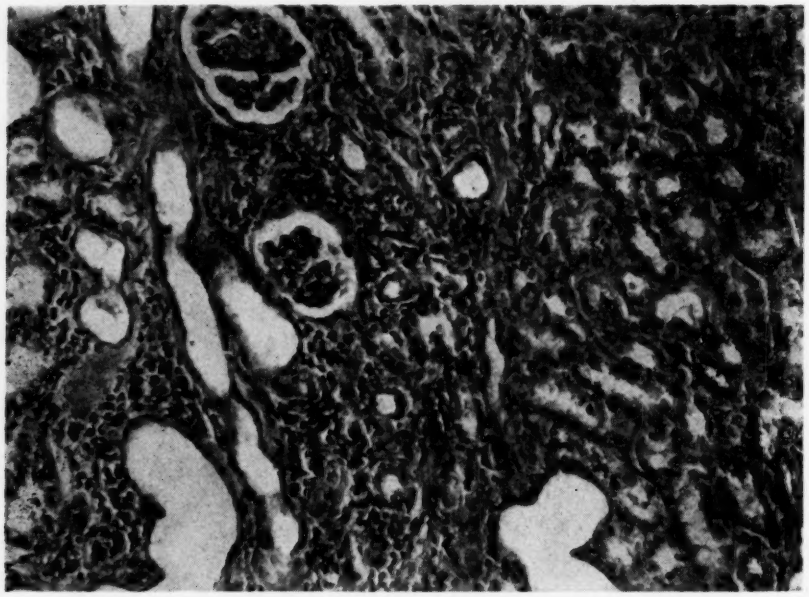
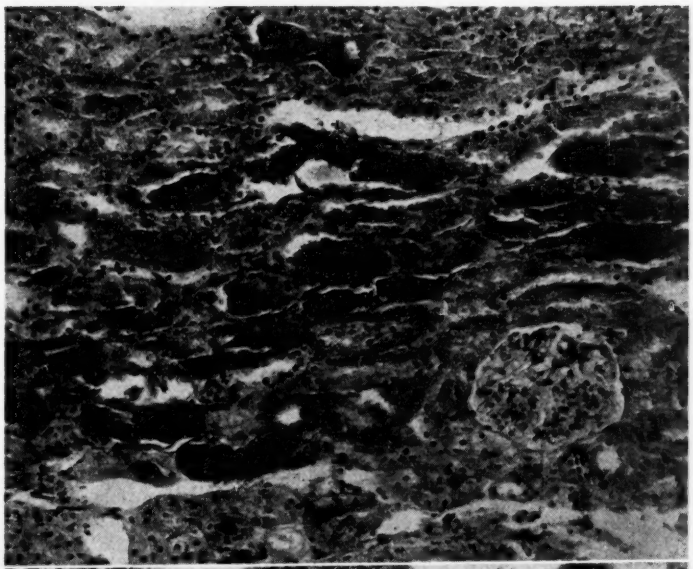


Fig. 5.—Kidney, Dog 8; alkaline urine; autopsy thirty-five days after injection of hemoglobin solution. Most of the cortex appears to be normal, but there are wedge-shaped areas of atrophy in which the tubules are shrunken or dilated and there are hemoglobin casts in the medullary tubules (×265).

At autopsy on the forty-sixth day the surfaces of the kidneys were finely pitted. On section it was evident that the pits were represented by radial streaks of atrophy. In an unstained section the medulla had brownish-red radial streaking. There were narrow wedges of normal cortex interspersed with areas of atrophy and casts. The lumina of the atrophic tubules were narrow but patent. Fig. 4 depicted an area of healed cortex adjacent to one of atrophy with large hemoglobin casts in the lumina of the atrophic tubules (forty-six days).

Fig. 6.



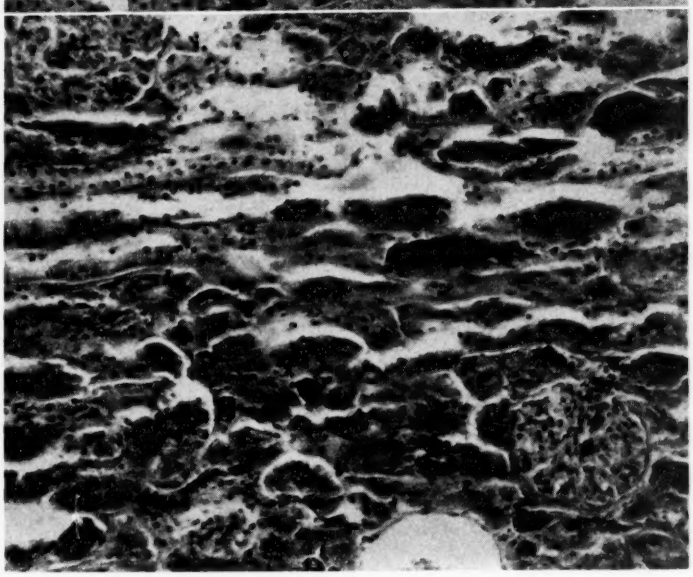


Fig. 7.

Fig. 6.—Kidney, Dog 4; alkaline urine; biopsy obtained five hours after injection of hemoglobin solution. A large number of hemoglobin casts are present in the loops of Henle and in the distal convoluted tubules. Some casts have a crystalline appearance, but others appear amorphous (×150).

Fig. 7.—Kidney, Dog 10; alkaline urine; biopsy obtained eight hours after injection of solution of hemoglobin crystals. There are numerous casts throughout the cortex and early degenerative changes (×150).

The findings in Dog 8 were similar qualitatively. The number of casts and the degenerative changes were less marked and the kidneys at autopsy showed much less permanent damage and casts than in Dog 3. Some of the atrophic tubules were dilated (Fig. 5). The renal function had returned practically to normal as evidenced by concentrating ability of the kidney. It is entirely possible that further repair of the anatomic damage would have occurred if the dog had been permitted to live.

The changes in Dog 4 were just as severe in the early stages as in Dog 8, but the progress toward complete healing was rapid after the eleventh day. Some pigment casts and atrophic tubules were evident in biopsies obtained at fifty-one and eighty-three days. At autopsy the kidneys were entirely normal with the exception of rare pigment casts and atrophic tubules. Fig. 6 from a biopsy obtained five hours after the hemoglobin injection revealed the very early appearance of casts in the tubules in spite of the continuous alkalinity of the urine. Experiments 4 and 8 illustrate the power of the kidney to regenerate after severe tubular damage.

In Experiment 10b the dog was given a solution made from 48.4 Gm. of crystalline hemoglobin. Thirteen per cent of the hemoglobin in this solution was methemoglobin. The pulse rate diminished to a low of 44 beats per minute six hours after the injection, and the pulse was weak. Twenty-four hours afterward the dog was listless and weak, but the pulse rate was 100 per minute and the pulse was strong. The dog was found dead forty-four hours after injection. Autopsy was not performed until fifty-two hours, or at least twelve hours after death. Unfortunately, considerable autolysis had occurred by this time. A biopsy (Fig. 7) obtained at eight hours revealed very many casts throughout the cortex. At autopsy the casts were more numerous. The tubule cells were badly degenerated (at least some of this represents post-mortem autolysis).

The results of Experiments 9 and 11 are so much alike that a description of the findings in Experiment 11 will suffice. Part of the explanted kidney in Dog 9 was replaced by an old hematoma. The hematoma probably was the result of trauma at the time of explantation, for there was troublesome bleeding from an artery over the lower pole. The hematoma may have been due to the initial biopsy.

In Experiment 9 hemolyzed red cells were used, but in Experiment 11 a solution made from crystalline hemoglobin was used. Dog 9 died on the seventh day and Dog 11 was killed on the tenth day, for it was obviously moribund. Each dog vomited after the third or fourth day but not before.

In Experiment 11, at twenty-four hours, there were many casts throughout the cortex and corticomedullary portion occluding the lumina of most of the loops of Henle and the distal segment. There were degenerative changes in the tubules not containing casts (Fig. 8). At three days degenerative changes were marked with desquamation of tubular epithelium, poor staining, and vacuolization of the cytoplasm and pyknosis of nuclei. Casts were very numerous. At six days the cells of the convoluted tubules were severely injured with more marked changes than noted at three days. At autopsy (ten days, Fig. 9) there

Fig. 8.

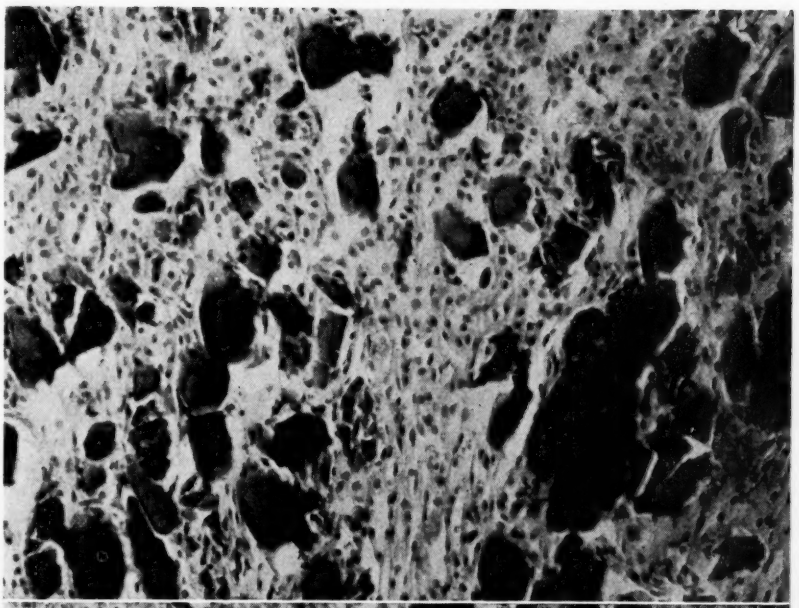


Fig. 9.

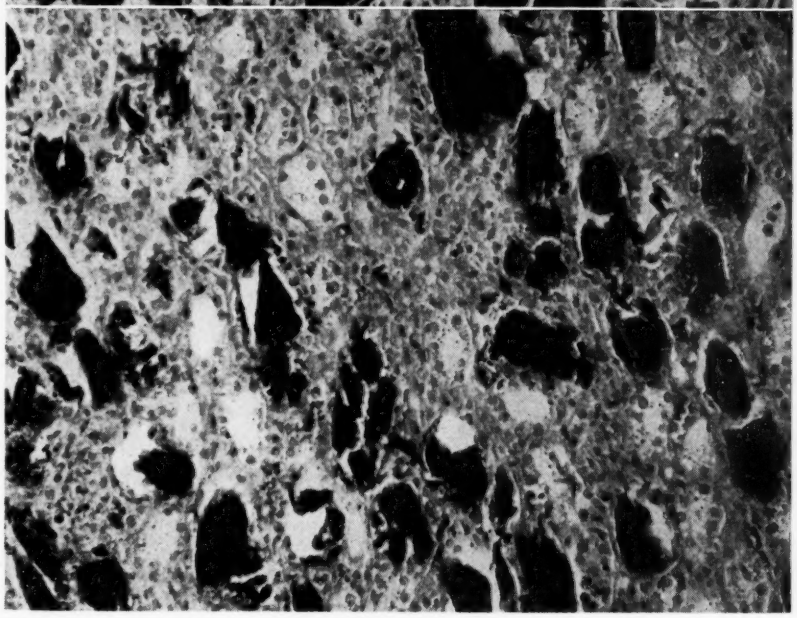


Fig. 8.—Kidney, Dog 11; acid urine; biopsy obtained one day after injection of a solution of hemoglobin crystals. The number of hemoglobin casts is the greatest of any of the experiments. There are degenerative changes in the tubules in the cortex (×265).

Fig. 9.—Kidney, Dog 11; autopsy ten days after injection of solution of hemoglobin crystals. The number of casts remains as large in the medulla and corticomedullary portions but is less in the cortex. Cortical tubules show degenerative changes progressing to atrophy of some and dilatation of others (×265).

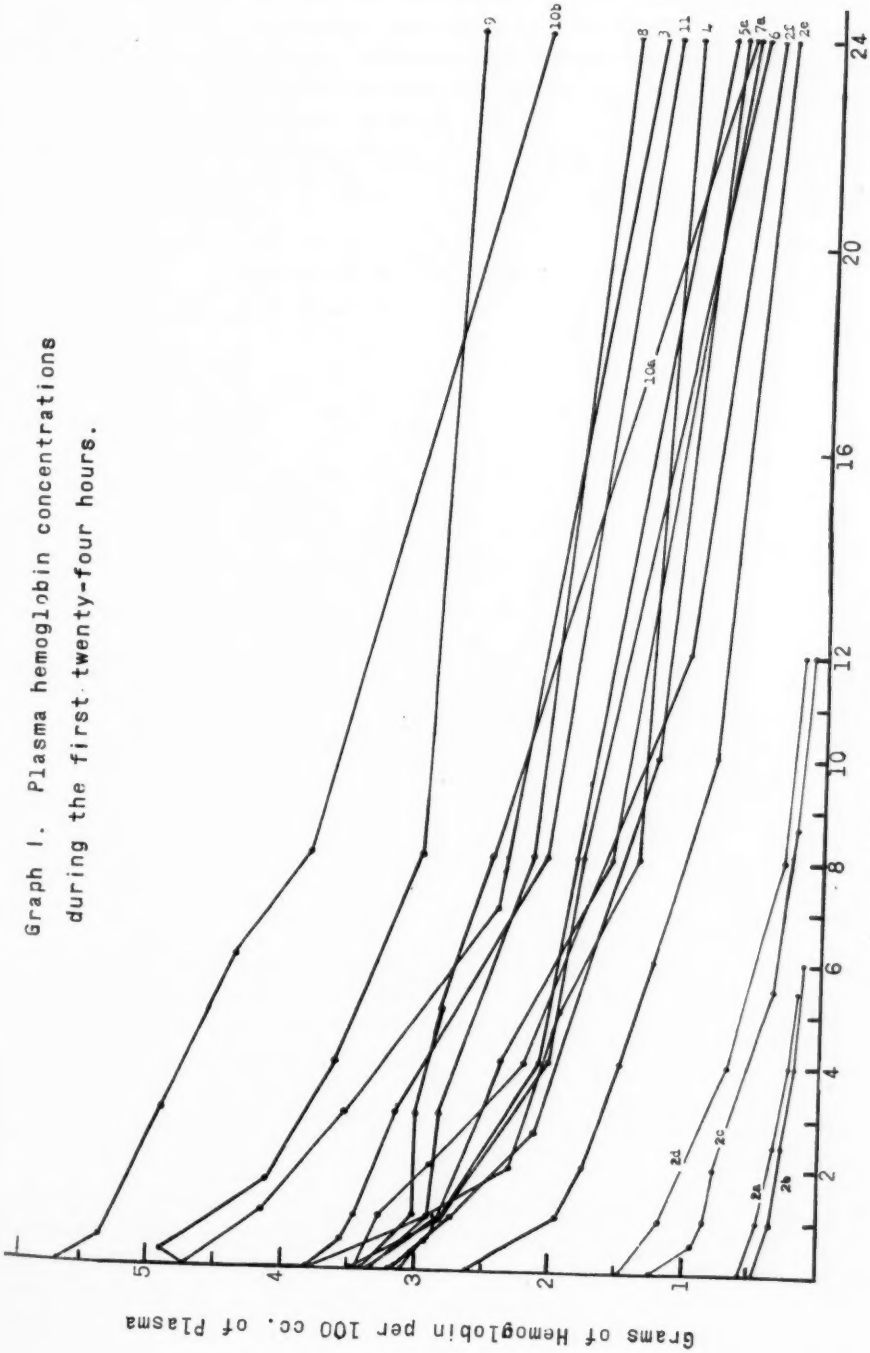
were many casts in the corticomedullary portion. In the cortex there was evidence of atrophy of some tubules, dilatation of others, and continued degeneration as previously noted. There was evidence of healing of some of the degenerated tubule cells. The explanted kidney had a small abscess at the lower pole, but the rest of the pathologic findings were identical in the two kidneys.

Microscopic examination of the urine was carried out at frequent intervals as indicated in Tables I and II. Casts described, unless otherwise specified, were large, dark brown, and granular. Some casts had square ends but most of them had a shape similar to any tubular casts. In Dog 11 casts appeared in the very first urine obtained after injection of hemoglobin but also in the urine of most dogs very soon after hemoglobinuria started. Because of catheterization the significance of hematuria is questionable.

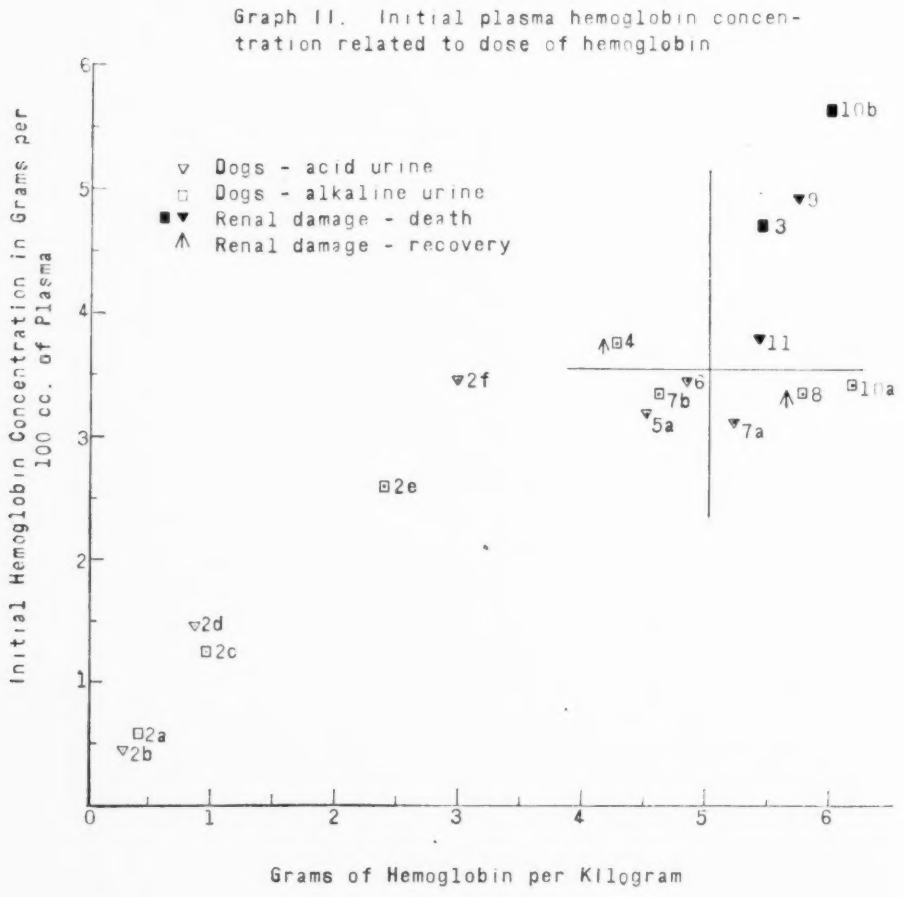
In Dogs 3, 4, 8, 9, 10a, and 11 retention of urea nitrogen, excretion of urine of low fixed specific gravity, albuminuria, and in three dogs, death from uremia are interpreted as evidences of renal insufficiency. There probably was an increased rate of protein metabolism in these dogs. When one subtracts the amount excreted, the dose of the administered hemoglobin did not constitute more than a large protein meal which any dog can eat in one day. It is doubtful that vomiting and dehydration had any significant role in these cases until development of frank renal insufficiency and uremia. Dogs 9 and 11 did not begin to vomit until uremia was well established. Dog 4 developed only transient renal insufficiency and probably could be classed with the other group, but it is classed in the group with renal insufficiency because of the histologic manifestation of moderate characteristic renal damage. No dogs developed anuria, but the dogs with renal damage continued to excrete rather large volumes of low specific gravity urine. One determination of the ratio of the urine urea nitrogen to blood urea nitrogen was made. It was found to be 455/155 on the fifth day in Experiment 11. This is further evidence of failure to concentrate urinary solids. Other workers[16, 31, 81] have observed the development of uremia in spite of excretion of normal or even large volumes of urine.

Graph I depicts the disappearance of hemoglobin from the plasma as evidenced by the plasma hemoglobin levels during the first twenty-four hours. It may be noted that there is a rather sharp decline in levels during the first four hours, but after that there is a more gradual and uniform decline. Hemoglobinemia continued for forty-eight hours or longer in all of the cases except in the experiments on Dog 2. (See Tables I and II for these details.) The duration of hemoglobinemia is directly related to the dose of hemoglobin administered.

There is a relationship between the initial plasma hemoglobin level and the dose of hemoglobin, but as illustrated in Graph II there are individual variations which cannot be readily explained on the basis of differences in hemodilution. One factor of importance is the concentration of the administered solution. In every experiment (except Experiment 11) in which the initial plasma hemoglobin level was above 3.5 Gm. per 100 c.c., the concentration of the solution administered was greater than 7.0 Gm. per 100 cubic centimeters. Dog 10 is a good example of this effect, for with comparable doses of hemoglobin the initial


Graph I. Plasma hemoglobin concentrations during the first twenty-four hours.
Grams of Hemoglobin per 100 cc. of Plasma
Hours after Completion of Injection
Graph I.

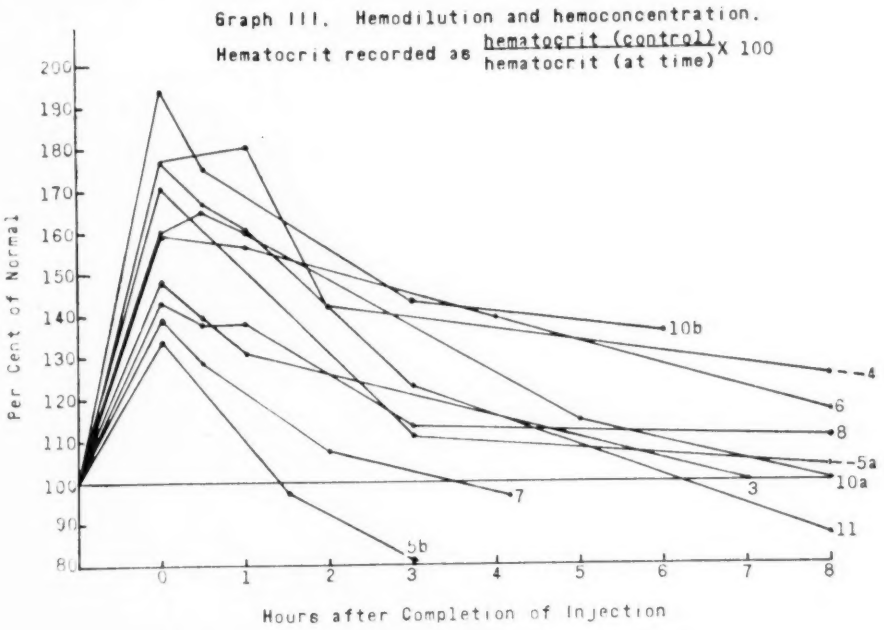
plasma level was 3.38 when the concentration of the solution was 4.10 Gm. per 100 c.c. and 5.63 Gm. per 100 c.c. when the concentration of the solution was 10.74 Gm. per 100 cubic centimeters. Of course, the factor of concentration is affected by the time necessary for administration and also by the total dose administered, so the effect of concentration probably is a combination of factors. Nevertheless, it is desirable to administer as concentrated a solution as possible in order to cause renal damage.



Graph II.

There are many factors affecting the disappearance of hemoglobin from the circulation. Graph III illustrates the constant occurrence of hemodilution in the experiments. The ratio of control hematocrit to hematocrit at the time is used, for it approximates the true extent of hemodilution. It may be noted that Dog 5 developed very definite hemoconcentration at three hours and died twenty minutes later. Dog 11 also developed a minor degree of hemoconcentration at eight hours, but the rest of the dogs either returned to normal or maintained some hemodilution at eight hours. When the blood for the control hematocrit

clotted, the experiment is not represented in the graph. The statement has been made that shock occurs in acute hemolytic conditions because of the sudden disappearance of a large volume of red blood cells,[37] but it must be remembered that the hemoglobin remains in solution for a time. Because of the osmotic effect of hemoglobin the volume of blood probably is maintained at its original level initially and only gradually decreases. Furthermore, the osmotic effect of hemoglobin probably limits the level of hemoglobin which can be attained by injection of solutions.



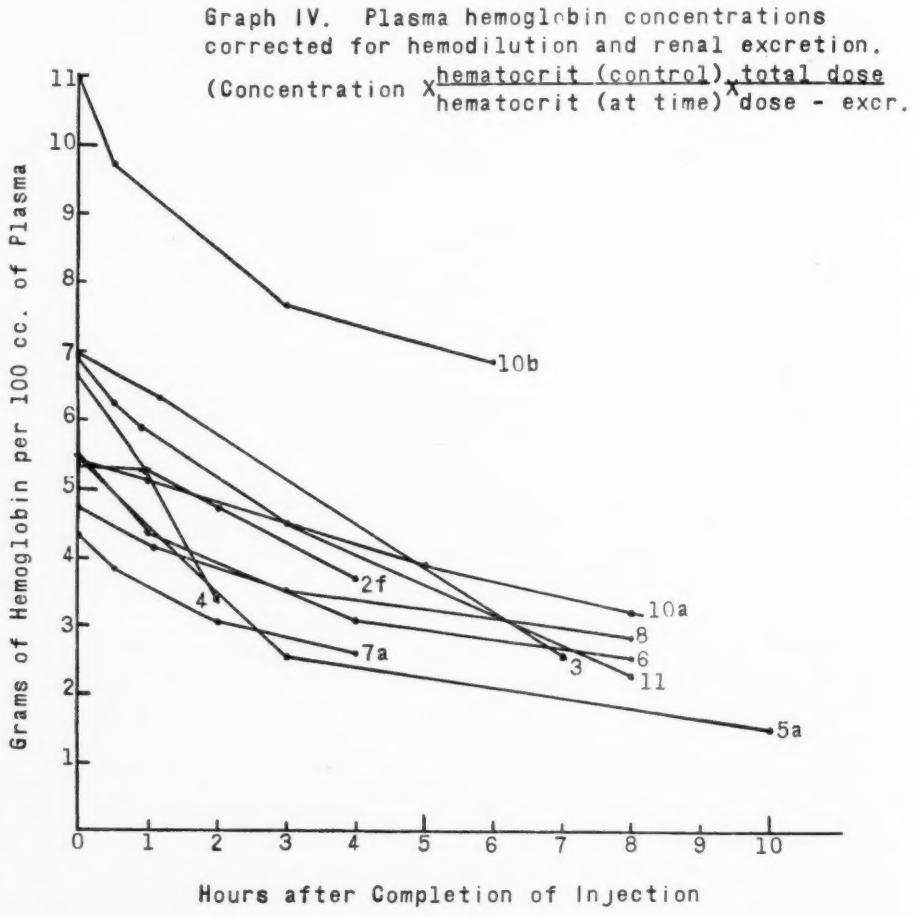
Graph III.

Splenic enlargement, which was evident on palpating the abdomen of all dogs immediately after completion of the injections, is also a manifestation of hemodilution. Another factor which cannot be evaluated accurately in the previously mentioned experiments is the occurrence of ascites with hemoglobin in the ascitic fluid. Ascites was present in Dogs 2 and 5 which died during the first eight hours of the experiments. In Experiment 5b it was possible to determine the hemoglobin level in the fluid a short time after the last plasma hemoglobin determination. The ascitic fluid level was only slightly lower than the plasma level. It is entirely possible that ascites was present during the early part of each experiment.

In order to eliminate or to compensate for the two major factors influencing the level of hemoglobin, namely, hemodilution and excretion of hemoglobin by the kidney, the plasma hemoglobin levels were multiplied by the ratio of control hematocrit to the hematocrit at the time and by the ratio of the initial dose of hemoglobin to the difference between the total dose and the amount excreted up

to that time. Graph IV depicts the approximately corrected hemoglobin levels. It is seen that there is a more uniform disappearance rate illustrated by this graph than by plotting the hemoglobin levels directly as in Graph I.

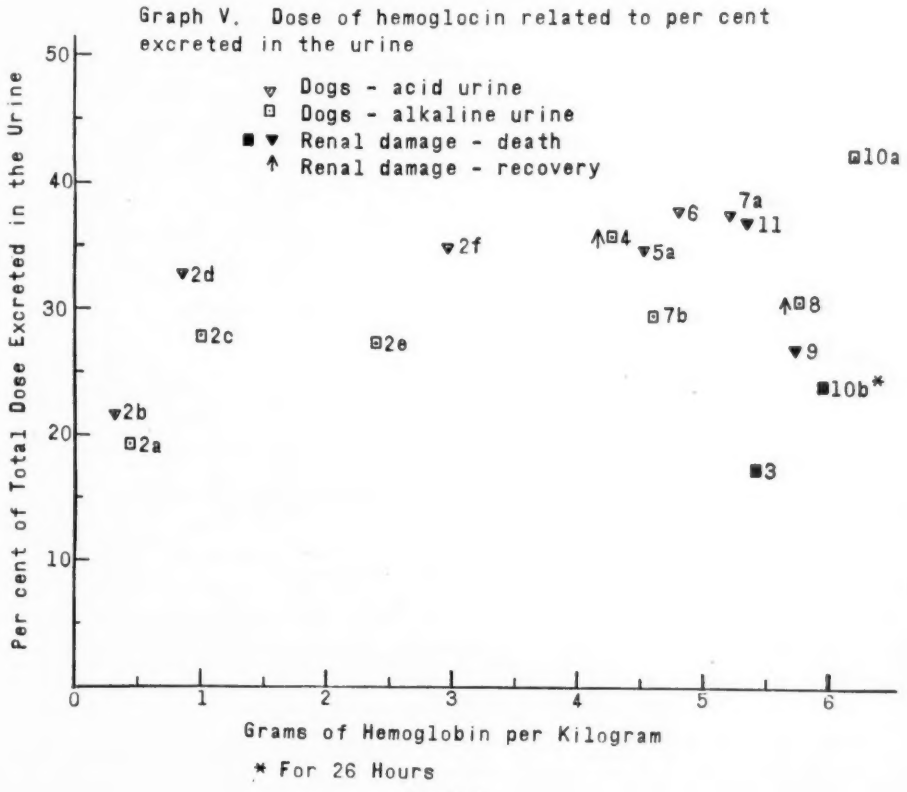
The body of the dog is removing hemoglobin from the circulation at a relatively constant rate. The correction for hemodilution is justified, for it gives the plasma hemoglobin level which would theoretically occur if all the hemoglobin were contained in the original blood volume. The correction for renal excretion is also justified if one is considering the ability of the organism to remove hemoglobin from the circulation, exclusive of the kidneys. The results in Experiments 4 and 10b cannot be explained readily.


Graph IV. Plasma hemoglobin concentrations corrected for hemodilution and renal excretion.
(Concentration X hematocrit (control) / hematocrit (at time) X total dose / dose - excr.
Grams of Hemoglobin per 100 cc. of Plasma
Hours after Completion of Injection
10b
2f
4
10a
8
6
11
3
7a
5a
0 1 2 3 4 5 6 7 8 9 10
1 2 3 4 5 6 7 8 9 10 11

Graph IV.

The amount of hemoglobin excreted is dependent on several factors. Graph V shows the scatter of per cent of hemoglobin excreted in relation to dose administered. It can be seen that there is an even distribution of dogs with acid urine and those with alkaline urine when large doses were administered. It may be seen also that the lowest and the highest percentage excretion were found in

dogs with alkaline urine when the larger doses were used. As might be expected, the dogs developing renal insufficiency excreted somewhat lower percentages than the other dogs. These findings do not agree with those of De Navasquez[25] who used much smaller doses in rabbits, but it is believed that these results are more significant since the doses used were sufficient to cause definite renal injury in about half of the dogs. The percentage of total dose excreted is in agreement with observations of other investigators.[43, 72, 73]

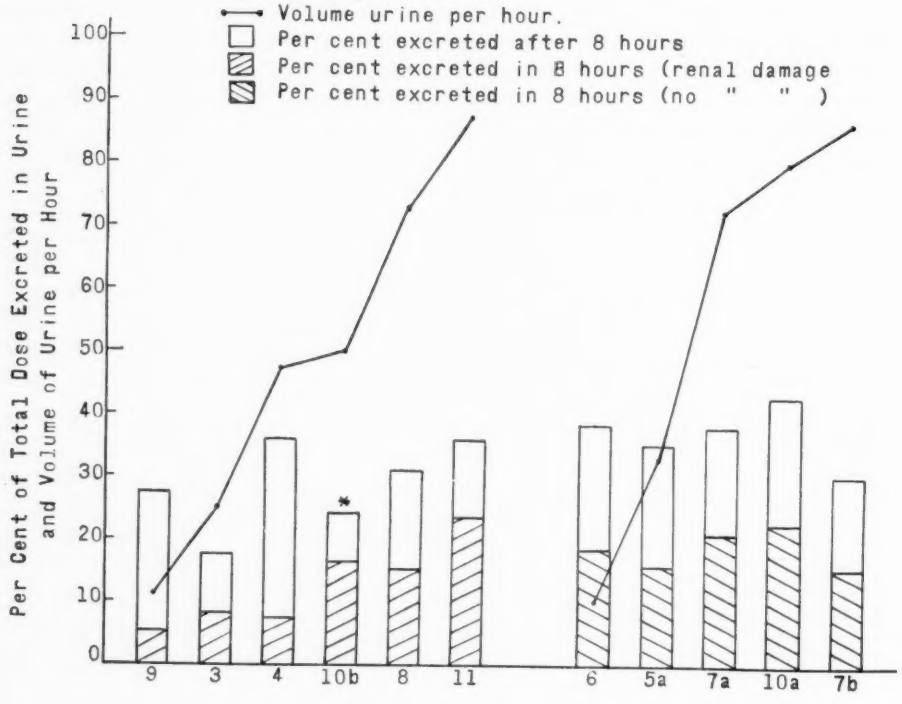


Graph V.

Graph VI depicts the relationship of hemoglobin excretion in the first eight hours to total excretion and to urine volume. The amount of excretion in the first eight hours is roughly related to the development of renal insufficiency, but there is enough overlapping of values that no generalization can be made. Notice the closely comparable curves of volume of urine in the two groups.

The plasma hemoglobin level of dogs given large doses of hemoglobin does not appear to be correlated very closely with the maximum urine concentration. It is noteworthy that in Experiment 2a the maximum urine concentration was ten times that of the plasma. The maximum concentrations in the urine occurred some time after completion of the injection and usually in the period from six to eight hours afterward. This may be related to available water for urine dilution.

Graph VI. Excretion of hemoglobin in per cent of initial dose and volume of urine in first eight hour period.



Graph VI.

DISCUSSION

The results obtained by explanting one kidney of a dog and using a Silverman biopsy needle have been satisfactory. It has been possible to obtain specimens for histologic examination of the kidney at as frequent intervals as considered useful during the course of the experiments previously described. That representative samples of the kidney are obtained has been demonstrated by comparing the biopsy with autopsy specimens of the same animal a short time later. Up to now the progress of a lesion of the kidney produced by hemoglobin or other chemical agents has been studied by sacrificing animals at intervals after introducing the noxious agent or by examining the animals as they died of renal insufficiency. The method of obtaining serial biopsies should be of considerable use in the further study of induced hemoglobinuria, renal damage by chemical poisons, and experimental renal hypertension.

There are some disadvantages to the method of needle biopsy. In two animals (Dogs 6 and 11) the explanted kidney had abscesses at one pole. Since none of the rest of the kidney or of the other kidney had evidence of suppura-

tion, it is reasonable to ascribe the infection to an introduction of bacteria by the needle. More care in scrubbing and preparing the skin probably would have eliminated one of the abscesses. In the other case the novocain solution was contaminated and probably caused the infection. Small areas of infarction and later scarring of the parenchyma occurred after some of the biopsies. Hematuria resulted in some instances, and it was usually possible to identify microscopically some epithelium of the pelvis in the biopsy when hematuria had occurred. There were no serious sequelae from the hematuria. The procedure was carried out occasionally without success in obtaining an adequate specimen. It can be done by a single individual, but help to compress the kidney afterward, to hold the dog, and to steady the needle makes the procedure easier and more certain of success.

It is suggested that a record of sites of biopsy be kept on a diagram of the kidney so that the same area is not used more than once. This was not done in any of the previously mentioned experiments, but some of the unsatisfactory biopsies could have been averted and less scarring of the kidney probably would have resulted.

In all dogs which developed renal insufficiency a number of casts persisted after cessation of hemoglobinuria. The most severe renal insufficiency occurred in the dogs with most marked accumulation of casts in the tubules (Experiments 9, 10b, and 11). Dog 3 developed severe renal insufficiency but had begun to recover some renal function when it died from pneumonia. The number of casts was almost as great as in the previously mentioned three instances. Dog 8 developed renal insufficiency lasting a shorter time than that of Dog 3. Dog 4 developed the mildest renal insufficiency and evidence of renal damage of the group. Again, the degree of insufficiency was correlated with the accumulation of casts in the cortex and medulla of the kidneys. The amount of tubular epithelial injury was directly correlated with the number of casts. In other words, hemoglobin casts and tubular epithelial injury are factors of equal significance in the production of renal insufficiency.

The specific gravity of urine in patients with blackwater fever is said to be variable and of no significance, but an examination of the protocols of patients dying from uremia[8, 95] reveals a fixed specific gravity of the urine even in the face of a severe oliguria. In burned patients with uremia following hemoglobinuria[81] normal or large volumes of urine with fixed low specific gravity were excreted. Ross[79] reported a group of patients with blackwater fever who never developed anuria and yet had renal insufficiency. Fairley and Bromfield[31] report a patient with blackwater fever who had polyuria from the start, developed uremia and acidosis, but eventually recovered.

Patients who have developed renal insufficiency as the result of any acute hemolytic episode may recover completely without any signs of renal damage on testing years later.[13, 45, 68] The recovery of animals after developing severe renal insufficiency has also occurred. In the present study it has been possible to follow the course of degeneration and subsequent healing or partial healing in Dogs 3, 4, and 8. Figs. 2 to 5 reveal the gradual process of healing from a

stage of severe injury (Fig. 2) to healing of some of the tubules and atrophy of others (Figs. 4 and 5). Less marked renal damage developed in Dog 4 and a longer period of observation was possible so that it had practically normal appearing kidneys 112 days after injection of hemoglobin. In some sections of the kidney in this dog an occasional atrophic tubule and rarely a cast could be seen but, for the most part, the kidney was normal. The appearance of the sections in Dog 8 is intermediate between those of Dogs 3 and 4. In Dog 11 which lived ten days after injection, there was evidence of beginning repair of the tubules of the cortex.

The histologic changes in the various experiments correspond with those reported by other investigators[6, 9, 59, 85] and to the photomicrographs published by DeGowin and co-workers.[24] It is noteworthy, though, that dogs which had distinctly alkaline urine developed renal insufficiency of all degrees of severity and that the histologic appearance cannot be distinguished from the dogs with acid urine which developed renal injury. This is at variance with the findings of DeGowin and co-workers. Furthermore, they found only minor degenerative changes in the tubular epithelium of most of their dogs dying from renal insufficiency. Their photomicrograph showing regeneration corresponds to findings in some of the experiments mentioned in the article. Some necrosis and desquamation of tubular epithelium was noted in the kidneys of a few of their dogs.

The histologic appearance of the kidneys of the previously described dogs developing renal insufficiency are very similar to the pathologic findings in clinical cases.[7, 21, 24, 45] Most of the reported cases of death from renal insufficiency occur on an average of seven to ten days after the initiating hemolytic accident, so that the findings correspond to those at the height of degenerative changes in the tubular epithelium and also at a time when the number of casts in the cortex seems to be diminishing in the dog experiments. When death occurs as early as twenty-four hours after the hemolytic episode, the tubule cells already show some degenerative changes. The appearance of hemoglobin casts in the renal tubule is a constant finding in the cases of hemoglobinuria with anuria in human beings and it is therefore pathognomonic of hemoglobinuria.

In reports of clinical cases the number of casts noted was often small. However, every section of a tubule need not be occluded by a cast, for only one small segment of a tubule need be obstructed theoretically in order to interfere with the function of the individual nephron. In a section of kidney parenchyma a single nephron is cut across in literally hundreds of places. Nevertheless, tubular cell injury is the predominant lesion microscopically in some transfusion reactions.[5, 24, 45] No data are available on the number of casts nor does it seem possible to obtain such data. It is true that the number of casts in the kidneys of human cases, especially of transfusion reaction, is less than found in some of the dogs of my experiments.

The identity of the manifestations, and the lesions caused by transfusion reaction on the one hand and those caused by intravascular hemolysis of the patient's own blood cells on the other, is very important. One does not have to

look for some strange effect from the transfused blood, such as foreign protein or allergic or isoagglutinin effects. The changes are attributable to the hemolysis itself and hence to the products liberated from hemolyzed red blood cells.

The type of solution does not alter the effect of hemoglobinemia. Severe renal damage was produced by a solution of hemoglobin made from hemoglobin crystals in two out of three dogs. With comparable amounts of hemoglobin (over 5.0 Gm. per kilogram) hemolyzed red blood cell solutions also produced serious renal damage in three out of four dogs. The crystalline hemoglobin solution was made according to the directions of Bing[11] who found no impairment of renal function as measured by creatinine and para-aminohippuric acid clearances but who gave much smaller amounts of hemoglobin. The renal damage produced by the two types of solution cannot be distinguished by histologic examination of biopsy or autopsy specimens.

The possible deleterious effect of potassium in the solution can be excluded in these dogs for two reasons. In the first place, Kerr[57] has shown conclusively that the potassium content of dog red blood cells is no greater for practical purposes than that of dog serum. In the second place, administration of a solution of washed crystals of hemoglobin would eliminate any soluble crystalloid.

The most important single index of the probability of development of renal damage in the dogs I studied was the initial plasma hemoglobin level (Graph II). All dogs which developed serious renal insufficiency had values above 3.7 Gm. per 100 c.c. immediately after completing the injection of hemoglobin. Only Dog 8 had an initial level less than 3.7 Gm. per 100 c.c. and it developed only moderate renal damage. An evidence of the importance of the amount of hemoglobin in causing renal damage is the fact that five out of seven dogs given more than 5.0 Gm. hemoglobin per kilogram developed renal damage. Dog 4 was given less than this amount and developed renal damage but had an initial hemoglobin level above 3.7 Gm. per 100 cubic centimeters.

Whether or not oxyhemoglobin alone is the responsible agent cannot be answered from these experiments. In Experiment 10b the methemoglobin concentration and the total hemoglobin concentration were determined in all plasma samples and in the original solution. The plasma methemoglobin levels ranged from 0.704 to 0.530 Gm. per 100 cubic centimeters. The hemoglobin solution administered contained 13 per cent methemoglobin. The methemoglobin could be demonstrated easily by spectroscopy in all plasma samples studied in this experiment. It should be noted that methemoglobin appeared in the urine immediately in this experiment, but in other dogs with alkaline urine it never appeared until after an initial period of five to ten hours of observation. In the other experiments neither the hemoglobin solution nor the initial plasma specimen had enough methemoglobin to be visible by spectroscopy. It is probable, however, that methemoglobin was present in amounts less than that required for spectroscopic identification. As little as 3 per cent of total hemoglobin pigments can be identified as methemoglobin by spectroscopy.[87]

Bing[11] concluded that methemoglobin was the toxic agent in hemoglobinemic states only when the urine is acid. The absence of renal damage in dogs without

acidosis does not account for the results in Experiment 10b nor in the other experiments on dogs with an alkaline urine. Methemoglobin has not been found to exist in the plasma of patients who have undergone a hemolytic transfusion reaction or developed blackwater fever.[32, 34, 39] Methemoglobin has been found in the plasma of patients with hemolytic crisis from sulfonamides,[37] but it is probable that the sulfonamide had a direct effect in the production of methemoglobin. It does not seem possible at present to decide what importance should be ascribed to methemoglobin as the noxious agent in hemolytic conditions. Unfortunately, quantitative determinations were not made in any but the last experiment; however, in this instance it was known that methemoglobin was present in large quantities by spectroscopic examination before injecting the solution. In previous experiments equally diligent search failed to demonstrate any methemoglobin by spectroscopy. Methemoglobin in the urine is not the cause of the renal insufficiency, per se. In Dogs 5, 6, and 7 (Experiment 7a) no renal insufficiency developed though methemoglobin was recognized in the urine from the time of the first few hours. On the other hand, methemoglobin did not appear in the urine of Dogs 3, 4, or 8 in the same period, in sufficient amount to be recognized spectroscopically, and yet renal damage occurred in these dogs.

There is some evidence from clinical cases to support the thesis that the plasma hemoglobin level and/or the amount of hemoglobin liberated are the important factors. Bordley[13] reviewed fifteen cases of hemolytic transfusion reactions. He found that the five patients who recovered received an average of 314 c.c. of blood. Ten patients who died received an average of 564 cubic centimeters. No patient receiving less than 350 c.c. died and none receiving more than 540 c.c. recovered. There have been reports of deaths occurring almost immediately after administering less than 100 c.c. of blood,[92] but such cases may well have a different significance than those developing renal insufficiency after transfusion reaction.

In cases of hemolytic transfusion reaction which I previously[36] reported the only patient who developed anuria was one who had the highest level of hemoglobin a day after the transfusions. In the protocols of patients with blackwater fever reported by Foy and Kondi,[39] Fairley and Bromfield,[31] and Yorke and associates[95] the patients with the highest plasma levels were the ones who developed oliguria and anuria. In the cases of hemolytic anemia with hemoglobinuria described by Fox and Ottenberg[37] one patient had a plasma level of 1.6 Gm. per 100 cubic centimeters. This patient died from pneumonia but had complete urinary suppression as a result of the hemoglobinuria. Another patient who died also had renal insufficiency. The plasma level of this patient was much lower than that of the patient with pneumonia and was comparable to levels in the two patients who recovered. No statement is made as to the time relationship of the individual values to onset of hemolytic anemia, so one cannot use the second case as evidence for or against the thesis.

Another important factor becomes apparent in the study of paroxysmal nocturnal hemoglobinuria. Whenever the plasma hemoglobin levels in patients

with this malady have been determined they have been relatively low. The highest recorded level which could be found was 288 mg. per 100 cubic centimeters.[52] Renal damage is not a feature of the disease.[93] It is probable that the amount of hemoglobin liberated is usually not sufficient to raise the level dangerously. Renal damage or death from anuria is not mentioned in a review of paroxysmal cold hemoglobinuria.[64]

The relationship of the reaction of the urine to development of renal insufficiency was the original purpose of the thesis. The original plan of conducting serial renal biopsies was to determine whether precipitation of hemoglobin occurs only when the urine is acid. The discussion of the pathologic findings in Dogs 3, 4, 8, and 10b is sufficient to refute this idea. The severest renal damage occurred in Dogs 9 and 11 with acid urine and Dog 10b with alkaline urine. Serious renal insufficiency and renal damage occurred in Dog 3 with plasma hemoglobin and total dose comparable to Dog 9; however, the dog was recovering but was still azotemic when it died of pneumonia at forty-six days. Dog 8 with an alkaline urine developed moderately severe renal damage and insufficiency when given a dose comparable to Dog 7 with an acid urine. Dog 7 did not develop renal damage. The urine in Experiment 5 was so nearly neutral that it probably should be excluded from the present discussion of relationship of urine reaction. Two animals with acid urine (Dogs 6 and 7a) and two with alkaline urine (Dogs 7b and 10a) did not develop renal damage from hemoglobinemia. From these experiments it is impossible to say categorically that alkalinizing the urine does not benefit any dog or that acidifying the urine does not harm any dog. Alkalinizing the urine certainly does not prevent the formation of hemoglobin casts in the kidney and does not prevent serious renal damage in all dogs. Under comparable conditions the dogs with acid urine and those with alkaline urine fared equally well or equally poorly, as the case may be. Severe and even fatal transfusion reactions can occur when the urine has been previously alkalinized.[14, 25] In crush injuries[17] the benefit of alkalinizing the urine has not been definitely established but may have merit.

The results of DeGowin and co-workers[23, 24] are not strictly comparable to these experiments. The dogs given the acid diet may have had urine with lower pH (pH values not recorded), and their dogs with alkaline urine actually had neutral or slightly acid urine at times and slightly alkaline urine at other times. Some of the dogs with alkaline urine developed uremia of considerable proportions but recovered. Another difference is that their dogs were not anesthetized.

Cohn and Edsall[19] have summarized the available information regarding the solubility of hemoglobin in relation to pH. The isoelectric point of hemoglobin is pH 6.6. The solubility of hemoglobin is at its minimum at this pH. Sodium and potassium chloride do not lead to a marked decrease in the solubility of hemoglobin or albumin in their saturated solutions. Sodium sulfate is one of the most effective salting out agents. Horse and dog hemoglobin are less soluble than human hemoglobin. This information, of course, does not take into consideration the possible effect of methemoglobin or hematin formation in acid

urine, but it is important to realize that hemoglobin is least soluble at about pH 6.5. Insufficient observations were made on the presence or absence of the manifestations of shock which may have occurred in some of the dogs. The influence of shock induced by continuous administration of histamine[28] for one-half to one hour is an interesting observation. No functional impairment of the kidney could be detected in spite of continuous anuria for the period of lowered blood pressure. Observations of the urine flow in the present study indicate that at no time during the first eight hours after injection did the blood pressure drop low enough to cause complete cessation of urine flow. Renal blood flow may have decreased enough to allow excessive concentration of urine in the tubules.

Yuile and co-workers[97] have recently reported results of some very important studies on the effect of preliminary ischemia on renal damage due to hemoglobin. They injected hemoglobin solutions in doses ranging from 1.25 to 2.32 Gm. into rabbits weighing from 2.15 to 3.2 kilograms. When the tubules of the kidneys had been previously damaged by a short period of complete ischemia or the administration of sodium tartrate, hemoglobin precipitated in the tubules of the damaged kidneys but not in normal kidneys. When the urine was acid hemoglobin casts were numerous and persistent and were associated with renal functional disturbances, in contrast to the lack of such disturbances when the urine was alkaline. These observations may have an important bearing on the puzzling problem of the relation of shock and anoxia to the development of the crush syndrome, "transfusion kidney," and related lesions.

SUMMARY AND CONCLUSIONS

A method of obtaining serial biopsies of the same kidney of a dog has been described. By explanting one or both kidneys to the subcutaneous space of the lumbar region the kidney becomes available for biopsy with a needle biopsy apparatus or for obtaining blood samples from the renal veins. Satisfactory biopsies were obtained. It is suggested that the technique may be used to advantage for studying other problems related to experimental injury of the kidney by various agents.

When 1 Gm. of hemoglobin or more per kilogram of body weight is injected into dogs, 25 to 40 per cent is excreted by the kidney. The remaining hemoglobin is destroyed in the retriculo-endothelial system or the circulating blood with the production of bilirubin and hemosiderin. When excretion and hemodilution are corrected for, the rate of disappearance of hemoglobin is found to be more uniform during the first eight hours than is apparent from the plasma hemoglobin concentrations alone. In other words, hemoglobin is destroyed at a fairly uniform rate by the dog.

The amount of hemoglobin excreted is approximately the same when the urine is alkaline as when it is acid. This is true with relatively large doses of hemoglobin and at comparable plasma hemoglobin concentrations. There are considerable variations in maximum concentrations of hemoglobin in the urine of dogs with comparable doses and plasma concentrations.

In all dogs which developed renal insufficiency, the following two pathologic findings occurred together: (1) A large number of hemoglobin casts appeared in the loops of Henle and distal tubular segments, especially in the corticomedullary portion. The casts persisted for a long time after cessation of hemoglobinuria. The majority of tubules were occluded by casts in dogs with serious or fatal renal damage. (2) Degeneration of the tubular epithelium occurred as early as four hours, reaching a maximum at five to seven days. If the dog lived longer, gradual regeneration with eventual return to normal architecture ensued. The results of the histologic observations agree with reports of other investigators, with the exception that dogs with alkaline urine had renal damage approximately as severe as those with acid urine.

The most important indices as to development of renal damage in the dogs observed were the initial plasma hemoglobin concentration and the average of the initial and twenty-four hour plasma hemoglobin concentrations. When the initial concentration was over 3.7 Gm. per 100 c.c. of plasma or the average of the two concentrations was over 2.2 Gm. per 100 c.c., renal insufficiency developed. Dogs with values below these levels all recovered without signs of permanent renal damage due to hemoglobinuria. The renal damage was just as severe when solutions of hemoglobin crystals were used as when centrifuged solutions of washed hemolyzed red blood cells were used in similar amounts and providing comparable plasma hemoglobin concentrations. The evidence obtained in this study did not offer support for Bing's belief that the renal injury is due to methemoglobin, neither did it wholly exclude this possibility.

None of the dogs developed anuria. Renal damage was manifested by an elevation of blood urea nitrogen, albuminuria, and excretion of urine of low fixed specific gravity. Casts were found in the urine during the period of hemoglobinuria in all dogs regardless of the urine pH. Casts were also found in the urine of dogs with renal damage after cessation of hemoglobinuria.

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THE TREATMENT OF PERNICIOUS AND RELATED ANEMIAS WITH SYNTHETIC FOLIC ACID

I. Observations on the Maintenance of a Normal Hematologic Status and on the Occurrence of Combined System Disease at the End of One Year

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THE synthetic *Lactobacillus casei* factor (pteroylglutamic acid), commonly known as folic acid, is acknowledged a potent agent in causing hematologic remissions in pernicious anemia and related macrocytic anemias.[1-8] These include the macrocytic anemias of sprue, pregnancy, and nutritional macrocytic anemia. Many clinical and biochemical problems arose following the initial hematologic experiments. The present study was undertaken in November, 1945, to answer two of the clinical questions: (1) Will folic acid control the anemia of pernicious anemia and sprue for long periods of time as successfully as liver extract? (2) Will folic acid prevent the development of the neurologic manifestations of pernicious anemia or control them after they have appeared? A long period of observation is required to obtain the answers. This article constitutes a progress report at the end of the first year of investigation.

MATERIAL AND METHODS

The twenty-eight subjects comprising the group studied were patients from the medical wards and dispensary, Cincinnati General Hospital, and patients referred to us by private physicians, usually because of sensitivity to liver extract. Twenty-one patients had pernicious anemia which had been controlled satisfactorily from two to seventeen years by injection of liver extract; three had pernicious anemia in relapse; two had sprue which had been poorly controlled by liver extract; one had nutritional macrocytic anemia; and one had macrocytic anemia and diarrhea secondary to an ileosigmoidostomy performed in 1937. Pernicious anemia was diagnosed by the following findings: macrocytic hyperchromic anemia, leucopenia, polymorphonuclear leucocyte hypersegmentation, thrombocytopenia, megaloblastic arrest of the erythrocyte series in the bone marrow, and absence of free hydrochloric acid in the gastric secretion after histamine stimulation on at least two occasions. The diagnosis of sprue was made by the presence of steatorrhea, flat glucose tolerance curve, hypocalcemia, macrocytic anemia, megaloblastic arrest of the erythrocytes in the bone marrow, free hydrochloric acid in the gastric juice after histamine, and absence of evidence for pancreatic insufficiency. The patient with nutritional macrocytic anemia had free HCl in the gastric secretions and a megaloblastic type of bone marrow and for many years had eaten a diet deficient in protein and vitamins of the B complex. Fifteen of the twenty-four subjects with pernicious anemia

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had had disease of the posterior columns, lateral columns, or peripheral nerves at some time in the past, but in only one instance was there any evidence of active neurologic disease when folic acid therapy was begun. Folic acid was given orally to each patient, usually in doses of 30 mg. three times a week but in several instances in doses as low as 10 mg. or as high as 50 mg. daily. Patients were visited in their homes to insure that the medication was taken as directed. All liver therapy was discontinued but no attempt was made to modify the usual diets of the patients in their homes. Those who were brought into the hospital in relapse or for special study were given a diet from which meat, liver, and glandular products were excluded.

Complete medical and neurologic histories were obtained and physical and neurologic examinations performed on each subject at the beginning of the study. The examinations were repeated every three months, or more often if the condition of the patient demanded it. Erythrocyte and white blood cell counts were done on peripheral blood with equipment certified by the United States Bureau of Standards. Hemoglobin was determined as acid hematin using the Klett photoelectric colorimeter; hematocrit determinations were made on oxalated venous blood (4 mg. potassium oxalate and 6 mg. ammonium oxalate per 5 c.c.) by the method of Wintrobe,[9] and reticulocyte and platelet counts were made by the wet technique using Dameshek's method.[10]. All hematologic tests were performed daily while the patients were hospitalized and biweekly or monthly while they were followed in the clinic. Sternal bone marrow aspirations were performed on all patients who were observed in hematologic relapse. Cover slip preparations for cytologic study of peripheral blood and bone marrow were stained with Wright-Giemsa stain.

Analysis of the gastric secretions after histamine stimulation was performed in each patient included in this study.

HEMATOLOGIC OBSERVATIONS

Seventeen of the twenty-one subjects with pernicious anemia in remission who received folic acid for approximately one year were maintained in a hematologic state equivalent to that achieved previously by adequate amounts of refined liver extract (Table I). In Cases 1 and 2 there was a fall in the erythrocytes and hemoglobin after nine to twelve months and in Cases 3 and 7 there was an increase. Minor fluctuations in the hematologic values occurred in all patients. Inspection of the statistical data for all twenty-one patients revealed no significant change for the group in erythrocytes and hemoglobin over the values at the beginning of folic acid therapy. Most of the patients noted an increase in appetite and weight. Three subjects with pernicious anemia in relapse (Cases 21, 23, and 24) had satisfactory initial hematologic responses following administration of 10 mg. folic acid daily. Two of the patients (Case 23, Fig. 1 and protocol and Case 21, Fig. 2 and protocol) were not maintained in remission on this dose, and as neurologic symptoms developed they required increasingly larger doses of folic acid to maintain the hematologic remission. The third patient with pernicious anemia in relapse (Case 24, Fig. 3 and protocol) treated with 10 mg. folic acid daily had a satisfactory hematologic remission following a peak reticulocytosis of 26 per cent on the eighth day and has maintained this remission for twelve months on 50 mg. daily.

The subject with nutritional macrocytic anemia (Case 27) received 10 mg. folic acid orally per day for four months. Reticulocytes rose to 11.6 per cent on the fifth day of treatment and erythrocytes and hemoglobin increased gradually from 2.6 millions per cubic millimeter and 14.3 Gm. per 100 c.c. to 4.7 millions and 15.7 Gm. in four months. Although folic acid therapy was discontinued

TABLE I. SUMMARY OF MAINTENANCE STUDIES WITH FOLIC ACID IN TWENTY-EIGHT PERSONS WITH MACROCYTIC ANEMIA; COMPARISON OF HEMATOLOGIC AND NEUROLOGIC STATUS OF EACH PATIENT AT BEGINNING OF EXPERIMENTAL PERIOD AND AT LATEST EXAMINATION

CASE	PATIENT	AGE	SEX	DIAGNOSIS	YEAR ESTABLISHED	U.S.P. UNITS LIVER EXTRACT PER WEEK BEFORE FOLIC ACID	FOLIC ACID DOSAGE (MG./WEEK)	MO. ON FOLIC ACID*	INITIAL ERYTHROCYTE COUNT (MILLIONS)	LATEST ERYTHROCYTE COUNT (MILLIONS)	INITIAL HEMOGLOBIN (GM./100 C.C.)	LATEST HEMOGLOBIN (GM./100 C.C.)	INITIAL HEMATOCRIT (PER CENT)	LATEST HEMATOCRIT (PER CENT)	COMBINED SYSTEM DISEASE		
															STATUS BEFORE LIVER EXTRACT	STATUS BEFORE FOLIC ACID	STATUS WITH FOLIC ACID
1	J. S.	56	M	Pernicious anemia	1934	10	90	13	4.30	3.25	14.5	12.3	43	40	Mild	None	No change
2	F. R.	75	M	Pernicious anemia	1935	10	90	13	4.50	3.32	13.9	12.8	41	38	Mild	None	No change
3	I. H.	70	F	Pernicious anemia	1929	10	90	13	3.91	3.86	11.4	13.0	39	40	Mild	None	No change
4	C. S.	56	M	Pernicious anemia	1944	60	90	12	4.82	4.81	15.5	14.7	49	48	Severe	Mild	No change
5	M. H.	55	F	Pernicious anemia	1943	10	90	13	4.47	4.54	13.0	14.8	45	43	Mild	None	No change
6	A. P.	46	M	Pernicious anemia	1944	10	90	11	4.62	4.96	16.3	17.7	51	48	Moderate	None	No change
7	E. W.	35	M	Pernicious anemia	1941	15	90	13	3.38	5.03	11.0	15.1	46	47	Mild	None	No change
8	R. P.	70	F	Pernicious anemia	1939	10	90	12	4.29	3.90	14.5	13.8	41	42	Mild	Mild	No change
9	M. S.	44	F	Pernicious anemia	1944	?	90	12	4.74	4.79	15.7	14.9	46	42	None	None	No change
10	D. K.	82	F	Pernicious anemia	1935	10	90	13	4.42	4.43	12.5	13.0	43.5	44	Mild	Mild	No change
11	R. H.	63	M	Pernicious anemia	1942	30	90	13	3.90	4.23	12.0	13.0	45	44	Mild	Mild	No change
12	N. C.	72	F	Pernicious anemia	1943	5	90	13	4.60	4.59	14.0	14.6	45	48	Mild	None	No change
13	N. T.	71	F	Pernicious anemia	1938	10	90	13	4.65	4.66	14.0	13.0	45	41	Severe	Moderate	No change
14	L. E.	67	F	Pernicious anemia	1935	30	90	13	4.74	4.57	14.5	13.7	40	42	Moderate	Mild	No change
15	L. W.	75	M	Pernicious anemia	1932	5	90	13	4.48	4.35	14.5	14.6	45	40	None	None	No change

16	A. K.	55	F	Pernicious anemia	1942	10	90	13	4.39	4.15	14.0	13.7	40	43	None	None	No change
17	R. S.	65	F	Pernicious anemia	1936	None for 1 yr.	90	6	4.11	4.72	14.8	15.3	43	47	None	None	No change
18	L. H.	63	F	Pernicious anemia	1938	10	90	13	5.20	5.18	15.0	14.6	50	46	None	None	No change
19	C. D.	62	M	Pernicious anemia	1938	3	90	13	4.50	4.83	14.0	15.0	46		Moderate	Stationary	No change
20	J. F.	65	M	Pernicious anemia	1940	?	90 ↓ 700	10+	3.65	3.50	12.9	13.9	37.5	39	None	None	Slowly progressive
21	M. V.	59	F	Pernicious anemia	1936	None for 9 mo.	70 ↓ 3500	8+	1.07	2.35	4.0	10.0			None	None	Severe
22	L. F.	62	F	Pernicious anemia	1935	7.5	90 ↓ 1400	9+	4.21	4.20	15.7	15.8	44	42	None	None	Slowly progressive
23	N. G.	63	F	Pernicious anemia	1946	0	35 ↓ 1750	6	1.57	3.57	7.2	12.8	19			None	Severe
24	F. C.	66	F	Pernicious anemia*	1945	0	70 ↓ 350	13	1.22	3.79	4.7	14.5	15.5	42	Mild? Neuritis	Stationary	No change
25	B. A.	64	M	Sprue	1941	15	90	13	5.00	5.16	15.3	17.4	51	51			
26	A. F.	35	M	Sprue	1944	10	630 ↓ 0	12	4.54	4.43	15.6	16.4	47	48			
27	J. I.	74	M	Nutritional macrocytic anemia	1946	0	70 ↓ 0	7	2.60	4.94	14.3	15.3	38	49			
28	D. J.	41	M	Secondary macrocytic anemia (ileosigmoidostomy)	1937	10	525 ↓ 105	7	4.37	4.28	16.5	14.6	47	46			

*Time of last blood counts and neurologic examinations.

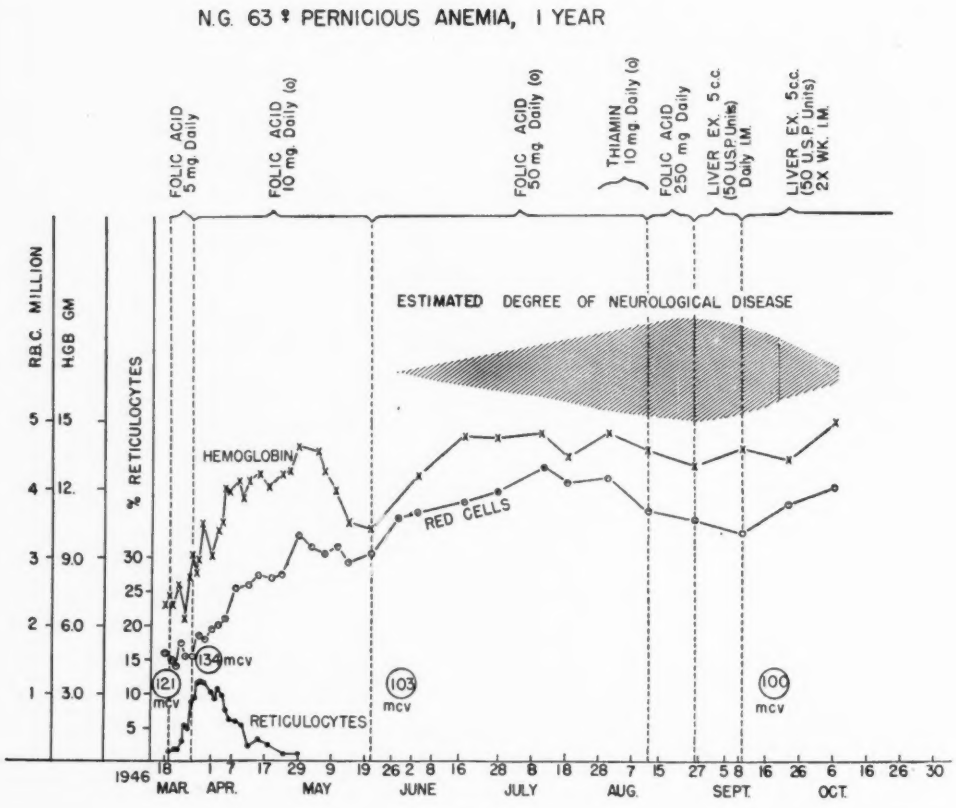
N.G. 63 ♀ PERNICIOUS ANEMIA, 1 YEAR
FOLIC ACID 5 mg. Daily
FOLIC ACID 10 mg. Daily (o)
FOLIC ACID 50 mg. Daily (o)
THIAMIN 10 mg. Daily (o)
FOLIC ACID 250 mg. Daily
LIVER EX. 5 c.c. (50 USP Units) Daily I.M.
LIVER EX. 5 c.c. (50 U.S.P. Units) 2X WK. I.M.
R.B.C. MILLION
HGB GM
% RETICULOCYTES
ESTIMATED DEGREE OF NEUROLOGICAL DISEASE
HEMOGLOBIN
RED CELLS
RETICULOCYTES
121 mcv
134 mcv
103 mcv
100 mcv
1946
18 MAR.
1 7 17 29 APR.
9 19 MAY
26 2 8 16 28 JUNE
8 18 28 JULY
7 15 27 AUG.
5 8 16 26 SEPT.
6 16 26 30 OCT.

Fig. 1.

Case 23.—N. G., a 63-year-old white woman, had anorexia and mild dyspepsia for a year and had lost 25 pounds in weight. There were no neurologic symptoms. The dietary excluded milk and eggs and the patient seldom ate meat.

Physical examination revealed only pallor of the mucous membranes, a waxy color of the skin, and a few red papillae at the tip of the tongue. There were no neurologic abnormalities.

Laboratory studies on March 19, 1946, showed: erythrocytes, 1.57 millions; hemoglobin, 7.2 Gm.; leucocytes, 4,950; reticulocytes, 1.6 per cent; platelets, 314,000; mean corpuscular volume, 121; mean corpuscular hemoglobin, 46; mean corpuscular hemoglobin concentration, 38; a normal leucocyte differential count; bone marrow, megaloblastic arrest of the erythrocyte series; normal gastrointestinal series; histamine refractory achlorhydria.

Folic acid orally, 5 mg. per day for eleven days, was begun on March 19, 1946. On the twelfth day, March 31, 1946, the dose of folic acid was increased to 10 mg. daily and maintained for approximately two months. In five weeks (April 25, 1946) the erythrocytes were 3.31 millions, the hemoglobin 13.8 grams. The patient felt entirely well, and although redness of the papillae of the tip of the tongue waxed and waned there was no soreness. The appetite was excellent and there was a weight gain of 22 pounds. After two months (May 19, 1946) hematologic values had fallen slightly and a neurologic examination showed: hyperactive knee and ankle jerks, positive Romberg sign, and slight reduction of vibration perception at the ankles. The only subjective complaint was of attacks of weakness in the right leg. The dose of folic acid was increased at this time to 50 mg. orally daily. There was a gradual

(Continued on opposite page.)

after four months, the patient's diet was improved to include foods rich in "extrinsic factor(s)." Three months after discontinuing folic acid there has been no relapse and none is to be expected.

The two subjects with sprue (Cases 25 and 26) were maintained in normal hematologic equilibrium. Remarkable improvement in diarrhea which had not been controlled by liver extract was noted in Case 26. Bowel movements decreased from five to six daily to one daily and weight increased from 127 to 147 pounds in two months. When folic acid therapy was discontinud for three months, mild glossitis appeared but cleared rapidly when folic acid therapy was reinstituted. The second subject with sprue (Case 25) continued in a satisfactory state of remission and the weight increased from 119 to 126 pounds.

The patient with macrocytic anemia secondary to ileosigmoidostomy (Case 28) was maintained in as normal a hematologic state by folic acid therapy as by crude and refined liver extract therapy. This person had had six to eight bowel movements daily since 1937 in spite of liver extract and was weak and distressed by abdominal distention. After three months of folic acid therapy the bowel movements had decreased to three daily, the weight had increased from 131 to 136 pounds, abdominal distention was partially relieved, and the patient was able again to play eighteen holes of golf without fatigue. (The improvement of a diarrhea essentially of a mechanical variety was not expected and no explanation is evident. Maintenance of a normal blood picture, however, suggests efficient absorption of folic acid from the small intestine.)

NEUROLOGIC OBSERVATIONS

Only one patient had subjective complaints or objective evidence of active central or peripheral nervous system degeneration prior to the administration of folic acid. This patient (Case 24, Fig. 3 and protocol) had numbness and

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rise in erythrocytes and hemoglobin during the next months (July 13, 1946) to 4.16 millions and 14.2 Gm., respectively. Within a week of the discovery of positive neurologic signs the patient noticed numbness and tingling in the hands and feet and unsteady gait. The neurologic signs and symptoms progressed rapidly in spite of a final increase in the dose of folic acid to 250 mg. orally daily for two weeks (Aug. 13, 1946). The patient was afraid to get out of bed for fear of falling. Numbness and tingling in the hands and feet were severe. Examination showed ataxia in arms and legs, hypalgesia over hands and feet, vibration perception 50 per cent reduced at the ankles and knees and 25 per cent in the fingers, and perception of position slightly reduced in the toes, deep reflexes hyperactive and the snout reflex positive; there were no plantar reversal signs or clonus.

At this point five months after continuous therapy with folic acid in increasing dosage and after a slight fall in erythrocytes and hemoglobin to 3.57 millions and 12.8 Gm., respectively, folic acid was discontinued (Aug. 27, 1946). Refined liver extract, 50 U.S.P. units, was administered intramuscularly daily for two weeks and twice weekly thereafter. In two weeks the patient felt much better. The paresthesias had almost disappeared. She walked well but with steppage gait. The Romberg test was normal, and there was no ataxia. Pain, light touch, temperature, and position perception were normal. Vibration perception improved but after six weeks of liver therapy was still reduced approximately 30 per cent at the ankles, 20 per cent at the knees, and 10 per cent in the fingers. The deep reflexes remained hyperactive. All redness disappeared from the tongue. The erythrocytes and hemoglobin rose very slowly in six weeks to 4.13 millions and 14.1 Gm., respectively.

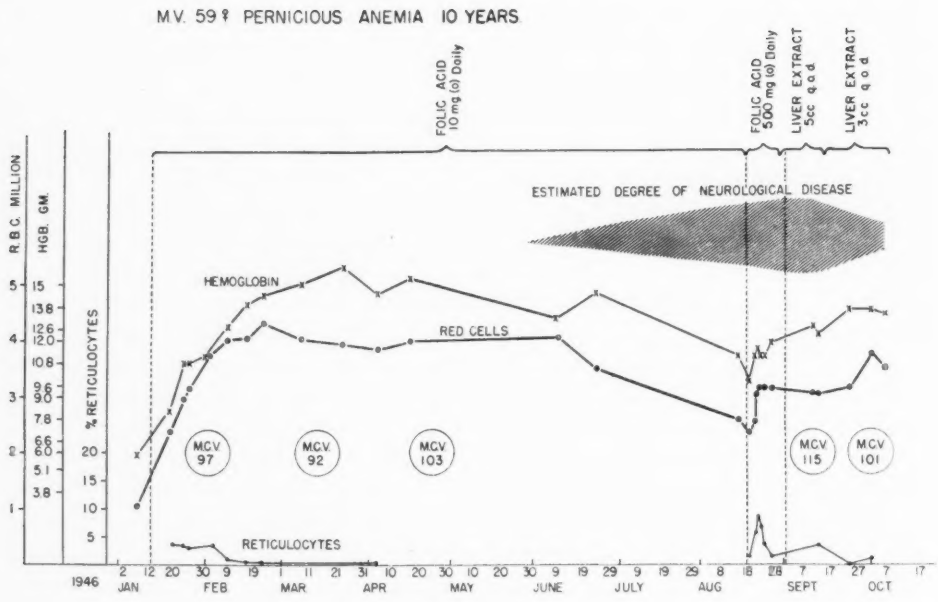

Fig. 2.

Case 21.—M. V., a 59-year-old white woman, had Addisonian pernicious anemia of ten years' duration. Treatment with liver extract had been sporadic because of hypersensitivity reactions. Symptoms of relapse were weakness, pallor, and anorexia. On Jan. 15, 1946, she had received a hypodermic injection which was said to be iron cacodylate.

The patient was small, thin, and pallid. Glossal papillae were atrophied. There was no evidence of brain, spinal cord, or peripheral nerve disease.

Laboratory studies revealed: histamine refractory achlorhydria; normal gastrointestinal series; erythrocytes, 1.07 millions; hemoglobin, "less than 6 Gm.," white blood cell count, 6,250.

Course: On Jan. 12, 1946, folic acid 10 mg. orally daily was begun. The blood picture improved and was well maintained on 10 mg. folic acid through the fifth month. At that time, however, stiffness in the knees and numbness and tingling in the fingers occurred. Examination showed: Romberg positive, ataxia in the legs, pain and light touch sensations preserved, position perception impaired in the toes, and vibration not felt at the ankles and approximately 75 per cent reduced at the knees. The patient left town without notice but had a supply of folic acid adequate for 10 mg. daily for two months. She returned at the end of this period unable to walk without assistance. She had evidently taken the folic acid but the exact amount could not be determined. Mental deterioration was obvious. The patient seldom completed a sentence and her memory was poor.

Examination showed: pallor, a few red papillae at the tongue tip; motor weakness at the ankles and knees; vibration perception absent at the knees and ankles, reduced in pelvis, normal in hands; perception of position of the toes absent; marked ataxia in the legs and ankle clonus; knee and ankle jerks hyperactive; and plantar reversal signs positive. Erythrocytes were 2.59 millions, hemoglobin 10 grams. The bone marrow showed moderate megaloblastic arrest in the erythroid series. The patient was hospitalized and given 250 mg. folic acid twice daily for twelve days. There was a moderate reticulocytosis to 8.6 per cent on the sixth day and a slight increase in erythrocytes but no change in the neurologic disease. Refined liver extract, 50 U.S.P. units daily for seven days and then every other day, induced neurologic improvement in ten days (disappearance of clonus and a normal Chaddock

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tingling in the hands and feet, stocking type of hypesthesia to touch, and pin prick and diminished knee and ankle reflexes, interpreted as signs of peripheral neuritis. While receiving folic acid, 10 mg. daily, the signs of peripheral neuritis increased. Remission or progression in these signs could not be correlated with increases or decreases in the dosage of folic acid and for the last nine months the patient's complaints and neurologic abnormalities have been essentially the same as when treatment was begun.*

After five to eight months on folic acid therapy, four subjects with pernicious anemia (Cases 20 to 23) noted the gradual onset of numbness and tingling sensations in the hands and feet, unsteadiness of gait, and moderate stiffness in the extremities. Neurologic signs appeared after the hematologic status had reached or was near normal and gradually progressed until there was definite evidence of combined system disease. The neurologic abnormalities implicated the posterior columns and peripheral nerves in four of the subjects, lateral columns in three, and cerebral cortex in one subject. None of these persons had neurologic involvement of any type when pernicious anemia was diagnosed initially or when folic acid therapy was begun. When signs of nervous system degeneration were unequivocal, usually indicated by the appearance of a positive Romberg sign, the extensor response on plantar stimulation, loss of sense of position and of perception of vibration in the feet, and hypesthesia and hypalgesia in fingers and toes, each of these four patients received 50 to 500 mg. folic acid orally per day for ten to forty days. These large doses of folic acid resulted in no subjective or objective improvement. Refined liver extract (10 U.S.P. units per cubic centimeter), 3 to 5 c.c. given intramuscularly per day, was begun after it was obvious that amelioration of the neurologic signs and symptoms would not occur or when progression of the disease was so rapid as to endanger the patient's future well-being. Within ten days thereafter improvement in the neurologic status began. The dose of liver extract was reduced to 3 to 5 c.c. on alternate days, and convalescence is progressing satisfactorily in all four patients. The progression of neurologic disease in the two patients most severely affected is indicated in Figs. 1 and 2. Shown in Fig. 3, Case 24, is the course of the patient with peripheral neuritis in whom the exacerbations and remissions seemed to occur independent of folic acid treatment. Accompanying each figure is the appropriate case history.

DISCUSSION

As yet too little time has elapsed to make sweeping statements regarding the effectiveness of folic acid as maintenance therapy for the patient with pernicious

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response). In two months she was clear mentally and could walk without aid but with a wide base. All plantar reversal signs had disappeared. There was slight swaying with the Romberg test. The position of the toes was perceived after maximal stimulation. Vibration was perceived at the knees but not at the ankles. The erythrocyte count and hemoglobin had gradually increased to 4.26 millions and 14.4 Gm., respectively.

*Since this article was submitted for publication this patient has developed definite posterolateral sclerosis, has been treated intensively with liver extract, and has shown a moderate remission.

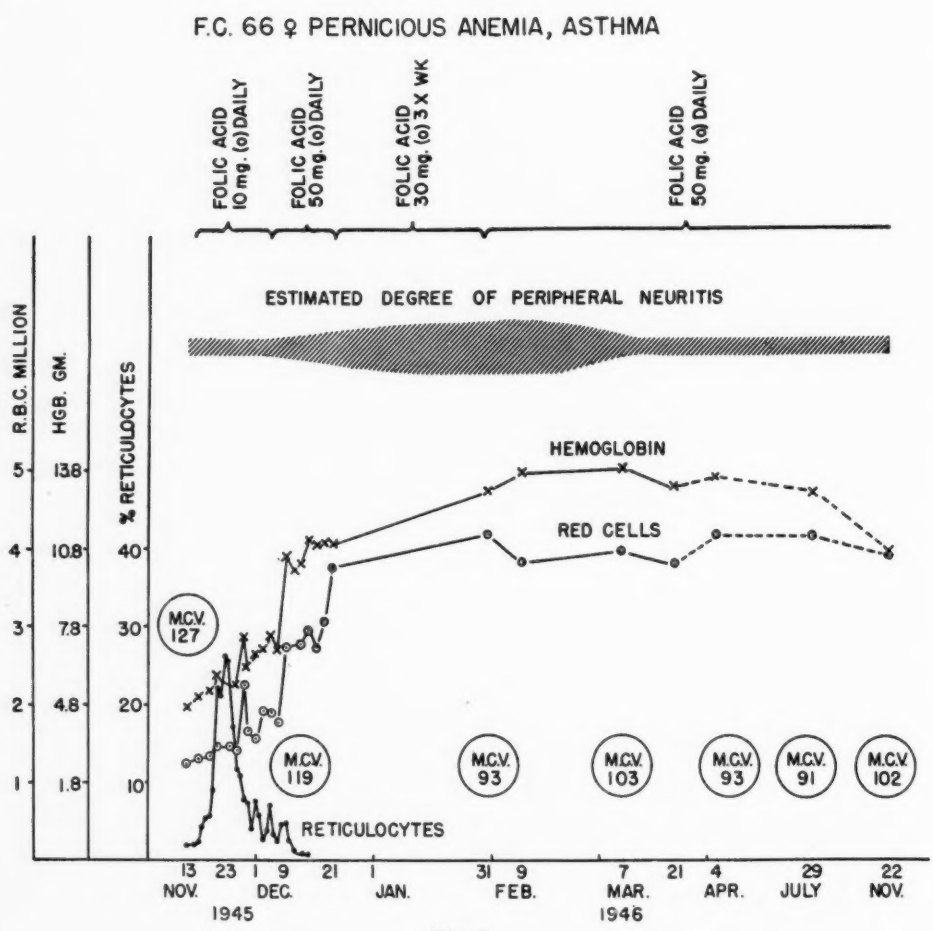

Fig. 3.

Case 24.—F. C., a 65-year-old white woman, complained of paresthesias in the hands and feet, intermittent glossitis, and progressive weakness for six weeks. Her two sisters had pernicious anemia. The patient was pale and slightly icteric. The tongue was normal. Vibratory perception was impaired at knees and ankles; perception of position of the toes was intact; there was hyperalgesia and hypesthesia on the palmar surfaces of the hands; and knee jerks were hyperactive and ankle jerks hypoactive.

Laboratory data revealed: erythrocyte count, 1.22 millions; hemoglobin, 4.7 Gm.; white blood count, 1,500; packed cell volume, 15 per cent; reticulocytes, 2.0 per cent; platelets, 178,120; mean corpuscular volume, 127; mean corpuscular hemoglobin, 38.6; mean corpuscular hemoglobin concentration, 30.4; megaloblastic arrest in erythrocyte series in the sternal marrow; and histamine refractory achlorhydria. X-ray studies revealed a small hiatus hernia and a duodenal diverticulum.

Synthetic folic acid, 10 mg. daily, was given orally beginning on Nov. 15, 1945. Reticulocytes began to rise on the second day and reached a peak of 26 per cent on the eighth day. In twenty days the erythrocytes and hemoglobin had risen to 1.90 millions and 7.5 Gm., respectively, and the dose of folic acid was increased to 50 mg. daily for seventeen days and then reduced to 30 mg. three times a week. In another ten days the erythrocyte count was 2.95

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anemia. It is impossible to predict how long a patient who has had adequate treatment with liver extract previously may go untreated without suffering a relapse.[11] The length of time before relapse depends upon such variables as the amount of stored erythrocyte maturation factor, the amount of intrinsic factor (however minute) produced by the stomach, the amount of extrinsic factor(s) in the diet, and the occurrence of infections and degenerative diseases. It is a well-known fact that adequately treated patients with pernicious anemia may suffer relapses within two to six months after liver extract is discontinued or they may remain in good health for as long as fifteen years.[12] Observations over a period of one year fall far short of being optimal; yet, considering the size of the group they suggest that folic acid will, in most cases, maintain the hematologic remission initiated by itself or by liver extract. There is no obvious mechanism to explain the fall in erythrocyte counts and hemoglobin levels in several of the patients. There was no evidence of infection or abnormality other than pernicious anemia in these patients and no obvious change in diets or activities. The amount of folic acid which previously had induced a remission in Cases 21 and 23 later seemed to be decreasingly effective in these same patients. Speculations on the mechanisms behind this are possible but too presumptive.

It is evident that folic acid did not prevent the occurrence of combined system disease, nor did massive doses halt or reverse the degenerative process once it had begun. Liver extract on the other hand bettered the neurologic status. These observations augment the single case of treatment failure of combined system disease reported by Moore[13] at the 1946 Meeting of the Society for Clinical Investigation and similar observations of Bethel[14] and Hall[15] and their associates. They confirm the many rumors that synthetic folic acid has proved disappointing in combined system disease. On the other hand, Doan[8] has reported one subject with mild neurologic abnormalities which disappeared during therapy with folic acid. The statement is made that "Minor neurologic signs and symptoms in patients in relapse have responded as promptly and completely following *L. casei* factor supplements as with potent liver extract, and no progressive cord lesions have as yet been noted."

Similar differences of opinion were recorded from 1927 to 1935, when the effect of liver extract in combined system disease was under close scrutiny.

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millions, the hemoglobin 11.1 grams. At this time (Dec. 17, 1945) there was proximal extension of hypesthesia in the hands and appearance of stocking hypesthesia in the legs. On Jan. 31, 1946, when the erythrocyte count was 4.19 millions and hemoglobin 13.0 Gm., further extension of signs of peripheral neuritis were noted. Folic acid was again increased to 50 mg. daily and continued through Nov. 20, 1946. In five weeks the signs of neuritis had returned to the initial degree. Repeated neurologic examinations revealed no change through November, 1946. No definite signs of posterolateral sclerosis had been observed at any time and the signs of neuritis appeared and disappeared independent of folic acid therapy. In September and October, 1946, a slight fall in the hemoglobin and erythrocytes was noted and on Nov. 22, 1946, the values were erythrocytes 3.9 millions, hemoglobin 10.7 grams. This occurred during an exacerbation of bronchial asthma and continued observation is necessary for final evaluations.

Careful studies during this period presented evidence for and against the beneficial effect of liver extract on the neurologic degeneration originally recorded by Minot and Murphy.[16] The effectiveness of liver extract was established only after many years of careful study and control of the potency. Therefore, final opinion must await further study of the effect of folic acid in various doses and by parenteral as well as oral routes of administration. Until a factor essential to the preservation of the central and peripheral nervous systems is found and until much more is known concerning the mode of action of folic acid in hematopoiesis, liver extract will remain the safest effective agent for the routine treatment of pernicious anemia.

SUMMARY

1. Folic acid in doses of 10 to 15 mg. daily or 30 mg. three times a week given orally maintained twenty-one persons with pernicious anemia in apparent hematologic remission for one year. Three patients with pernicious anemia in whom treatment was begun during a relapse responded satisfactorily to an initial daily dose of 10 mg. folic acid, but two of them required increasing amounts daily to maintain the remission; the third patient received 50 mg. daily for most of the year to determine the effect of the drug on peripheral neuritis.

2. Folic acid given orally controlled the manifestations of sprue in two persons for one year, nutritional macrocytic anemia in one person for seven months, and macrocytic anemia secondary to ileosigmoidostomy in one person as/or more effectively than liver extract.

3. Folic acid in doses of 10 to 50 mg. daily did not prevent the development of combined system disease in four persons with pernicious anemia. After combined system disease developed, folic acid in doses of 100 to 500 mg. daily given orally did not stop its progression in these persons. Liver extract, however, promoted rapid improvement.

4. These observations suggest that an unknown factor or factors in liver other than folic acid are essential for maintaining the integrity of the central and peripheral nervous systems in persons with pernicious anemia.

5. Folic acid cannot be considered satisfactory or complete treatment for pernicious anemia and should not replace potent liver extract in the routine treatment until another method for the protection of the nervous system is devised.

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STUDIES OF THE DISTRIBUTION OF INTRAVENOUSLY ADMINISTERED COLLOIDAL SOLS OF MANGANESE DIOXIDE AND GOLD IN HUMAN BEINGS AND DOGS USING RADIOACTIVE ISOTOPES

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INTRODUCTION

RECENTLY in the course of internal radiation therapy using colloidal manganese dioxide containing radioactive manganese (Mn^{52})[1] an opportunity has been presented to study the distribution of small particles in human beings when introduced intravenously. Similar observations have been made using colloidal metallic gold containing the radioactive isotope Au^{198}. In several patients whose disease came to fatal termination it was possible to determine the radioactivity of various tissues taken at autopsy, permitting a relative comparison to be made of their concentrations of manganese or gold. Three patients also were studied whose life expectancy was a matter of hours. Here only tracer doses were given. One of these subjects received the material via the intraperitoneal route. The distribution studies in human beings were augmented by studies of distributions in dogs; a record was obtained also of the disappearance of colloidal gold from the circulation in one dog and in one human subject.

RESULTS

In Table I is shown the relative distribution of radioactivity in selected tissue samples from eleven patients who had received radioactive manganese and two who received both manganese and gold. The distributions in patients receiving both isotopes were determined under conditions where the radiation from gold had disappeared. Thus only manganese is measured. Usually several doses had been given; however, due to radioactive decay, conditions were such that almost all of the radiation was due to a particular final dose. Since all values are relative some method of common comparison is required. Thus we have expressed the results in terms of the ratio of the activity per gram of tissue (wet weight) to that of the heart (muscle). Patient 6 is of particular interest since the dose was given intraperitoneally to this subject. In Table II are shown equivalent data for two patients who received gold alone. We attribute the approximate tenfold difference in numerical results primarily to a large variation in the content of the heart muscle in the two patients. In one patient we were able to determine tissue concentrations in micrograms of gold per gram of tissue. It was possible also to estimate the percentage of the dose

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*Now in the United States Navy Medical Corps.

TABLE I. RELATIVE DISTRIBUTIONS OF MANGANESE IN HUMAN BEINGS

ORGAN	PATIENT												
	6+*	4+	9	11	3+	2	7	12°	8	10	13°	5	1
Liver	6.4	7.3	20.0	53.0	17.0	32.4	69.0	43.0	15.6	17.6	7.5	46.0	15.6
Spleen	0.5	3.3	2.2	6.0	2.5	1.2	2.7	15.0	1.6	–	2.2	2.5	1.0
Heart (ventricle)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Heart (auricle)	–	–	–	–	–	1.4	–	–	–	–	–	–	–
Lung (right)	0.3	0.6	2.6	0.65	0.3	1.6	3.0	–	–	–	0.54	–	–
Lung (left)	0.6	0.6	–	–	0.5	–	–	–	–	–	–	2.2	–
Vertebral marrow	–	3.2	–	0.28	0.5	1.9	–	–	–	1.0	–	0.2	–
Pancreas	0.8	4.9	9.4	41.0	3.5	14.5	31.0	13.0	11.3	–	7.7	29.0	–
Adrenal	2.0	–	–	2.1	5.5	4.0	–	–	3.2	12.0	1.0	–	–
Testis	–	–	–	0.9	–	–	–	–	1.0	–	0.9	1.8	–
Kidney (right)	1.8	3.9	12.1	59.5	–	9.7	41.0	3.0	10.5	–	3.1	11.6	10.6
Kidney (left)	1.7	3.1	–	–	1.5	–	–	–	–	–	–	11.6	–
Muscle (striated)	0.2	–	–	–	–	–	–	–	–	–	–	1.3	–
Lymph nodes:													
Pancreatic	–	–	–	–	–	4.4	–	–	2.0	–	–	–	–
Mesenteric	1.9	1.9	2.2	3.4	–	3.5	19.6	–	–	–	–	–	–
Perihepatic	–	–	–	–	–	–	–	–	2.0	–	–	–	–
Retroperitoneal	–	1.9	5.8	–	–	–	–	–	–	–	–	3.7	–
Periaortic	–	–	–	–	3.2	–	–	–	–	–	–	–	–
Hilar	–	4.4	3.1	–	4.5	–	–	–	0.5	–	–	–	–
Omental	–	–	–	–	3.9	–	–	–	–	–	–	–	–
Mediastinal	–	–	–	–	2.9	–	–	–	–	–	–	–	–
Inguinal	–	–	–	–	–	–	–	–	–	–	–	5.5	–
Perisplenic	–	–	5.3	–	–	–	–	–	–	–	–	–	–
Brain	–	–	–	–	0.1	–	–	–	–	–	–	–	–
Stomach	–	–	–	–	–	5.5	–	–	–	–	–	2.4	–
Jejunum	0.4	–	2.1	–	0.7	–	6.6	–	–	–	–	1.5	–
Ileum	–	–	4.2	–	–	–	–	–	–	–	0.23	1.2	–
Colon	–	–	1.8	–	–	–	–	–	–	–	–	–	–
Uterus	–	–	3.0	–	–	–	–	–	–	–	–	–	–
Fat:													
Omental	–	–	–	–	0.9	–	–	–	–	–	–	–	–
Perisplenic	–	–	–	–	–	–	–	–	–	–	–	1.5	–
Perinephric	–	–	–	–	–	–	–	–	–	–	–	0.8	–

Values are in counts per minute per gram of tissue relative to heart muscle as unity.
Patients are arranged in order of the increasing time between final dose and autopsy.
+, Tracer amount only.
°, Patients received both Mn and Au; only Mn counted.
*, Injected intraperitoneally.

given which remained in the liver, spleen, and lungs. Variations in concentration throughout a given organ make this estimate rather uncertain. This effect becomes evident upon inspection of the data in Table III, in which studies of gold distribution in four dogs are presented. The large variation in the estimated percentage of the dose retained by the liver is evident. In Dog 4 the concentration in liver and spleen is determined from multiple samples, and the data show a consistent picture of complete removal of gold by these organs alone. The variation in gold content of different samples of liver and spleen in Dog 4 is shown in Table IV. The distribution of manganese in liver, spleen, pancreas, and heart of two dogs is shown in Table V. Here, instead of determining the activity of random samples, entire organs were used.

TABLE II. DISTRIBUTION OF COLLOIDAL GOLD IN HUMAN BEINGS

ORGAN	PATIENT 14 RELATIVE ACTIVITY	PATIENT 15 RELATIVE ACTIVITY	PATIENT 15 GOLD CONCENTRATION	PATIENT 15 ESTIMATED FRACTION OF INITIAL DOSE
Liver	28.6	800	0.76	60%
Spleen	93.0	430	0.41	3%
Heart	1.0	1.0	0.00095	-
Pancreas	-	1.4	0.0013	-
Kidney	26.0	1.9	0.0018	-
Marrow:				
Vertebral	11.0	-	-	-
Sternal	-	120.0	0.11	-
Lymph nodes:				
Axillary	2.0	-	-	-
Mesenteric	-	0.95	0.0009	-
Cervical	-	-	-	-
Periaortic	2.9	-	-	-
Lungs	3.1	46.0	0.044	2%
Adrenal	5.1	2.0	0.019	-
Testis	6.2	0.7	0.00066	-

In Patient 14 results are expressed in counts per minute per gram of tissue relative to heart muscle taken as unity.

In Patient 15 concentrations in micrograms per gram of tissue are given and estimates of the total fraction of the initial dose in certain organs are calculated.

THE COLLOIDAL SOLS

The manganese dioxide dispersions were prepared by oxidizing manganous chloride with potassium permanganate. Gelatin was used as the supporting colloid. The method has been described previously in more detail.[1] The sols were not dialyzed and there is some evidence that a small fraction of undispersed crystalloidal manganese is present.[2] Although precise determination of particle size was not made in each case, frequent microscopic observations of these dispersions using dark-field illumination showed that they were ultramicroscopic in size and exhibited a vigorous Brownian movement. In one case a sol was prepared for electron microscopy and a picture was taken.* The distribution in size of the particles of this sol was determined from the electron micrographs and is shown in Fig. 1. The second maximum may be due to the interaction of excess permanganate with gelatin. In all cases these sols had a deep brown color when observed by transmitted light and a greenish cast under reflected light.

In the animal experiments quantities of the order of 5 mg. of gold or less could be dispersed with good control of particle size. The sols were prepared by reducing a solution of gold chloride with ascorbic acid. Due to the instability of ionic gold and the presence of excess ascorbic acid, the gold dispersal should certainly be complete. Although gelatin was used, it is not essential. At pH 3 to 5 a characteristic dispersion was obtained containing relatively large particles. Such a sol was pale blue by transmitted light and had a heavy brick-colored blush under reflected light. The Tyndall beam was strong and an appreciable fraction of the scattered light was unpolarized. Under dark-field illumination the particles exhibited a relatively sluggish Brownian motion. The

*Electron microscopy was kindly done by Dr. H. C. O'Brien, Department of Physics, University of Pittsburgh.

TABLE III. DISTRIBUTION OF COLLOIDAL GOLD IN DOGS

ORGAN	DOG 1			DOG 2			DOG 3			DOG 4		
	A	B	C	A	B	C	A	B	C	A	B	C
Liver	2.9	2700	27%	7.3	1300	66%	11.7	25000	101%	11.5	24000	92.5%
Spleen	7.3	6800	7%	2.8	490	2.5%	1.5	3200	2.5%	10.3	21400	8.3%
Lung	0.54	50	2%	0.056	1	-	0.11	230	-	0.025	5.2	-
Heart	0.0107	1	-	0.057	1	-	0.0047	1	-	0.0048	1	-
Pancreas	0.010	0.93	-	0.0011	0.019	-	0.0022	0.47	-	0.001	0.21	-
Brain	0.0019	0.18	-	0.0004	0.007	-	0.0004	0.08	-	-	-	-
Kidney	0.072	6.7	-	0.016	0.28	-	0.013	28.0	-	0.0057	1.2	-
Gut	0.0019	1.8	-	0.0075	0.13	-	0.004	0.85	-	0.0016	0.33	-
Thyroid	0.0078	0.73	-	0.0056	1.0	-	0.0044	0.94	-	-	-	-
Adrenal	0.053	0.50	-	0.029	0.51	-	-	-	-	-	-	-
Marrow:												
Vertebral	0.085	7.9	-	0.044	0.77	-	0.043	9.1-	-	-	-	-
Sternal	0.020	1.9	-	0.059	1.0	-	0.096	20.0	-	-	-	-
Long bone	0.15	14.0	-	0.11	1.9	-	0.24	51.0	-	-	-	-
Lymph nodes:												
Mesenteric	0.012	1.1	-	0.0074	0.13	-	0.024	5.1	-	0.0096	2.0	-
Cervical	0.023	2.2	-	0.029	0.51	-	0.0093	2.0	-	-	-	-
Axillary	0.023	2.2	-	0.0031	0.054	-	0.012	2.6	-	0.002	0.42	-
Inguinal	-	-	-	0.0023	0.04	-	-	-	-	0.002	0.42	-
Ovaries	-	-	-	0.0047	0.082	-	-	-	-	-	-	-

A, Values are in micrograms of gold per gram of tissue.
B, Values are ratio of concentration to that of heart ventricle.
C, Values are estimated fraction of initial dose in organs of high uptake.

TABLE IV. VARIATION IN GOLD CONCENTRATION IN LIVER AND SPLEEN OF ONE DOG

LOCATION OF SAMPLE	CONCENTRATION
Liver—tip of right central lobe	8.0
Liver—center of right central lobe	11.6
Liver—top of right central lobe	16.1
Liver—center of lobus quadratus	10.7
Liver—center of left central lobe	13.3
Liver—center of left lobe	12.6
Liver—center of left lobus Spigelii	12.6
Liver—center of right lobe	11.4
Spleen—extreme dorsal end	9.9
Spleen—one-third of total length from dorsal end	9.0
Spleen—one-third of total length from ventral end	11.2
Spleen—extreme ventral end	13.1

The notation is that of Ellenburger and Baum.[3]
Concentrations are in micrograms of gold per gram of tissue.
All spleen sections, 1 cm. thick, are taken transverse to the major axis of the organ.

TABLE V. DISTRIBUTION OF RADIOACTIVE MANGANESE IN DOGS

	DOG 5			DOG 6		
ORGAN	ACTIVITY	PER CENT INITIAL DOSE	RATIO TO HEART	ACTIVITY	PER CENT INITIAL DOSE	RATIO TO HEART
Liver	11.8	55.0	11.0	9.6	48.0	12.0
Spleen	1.7	1.0	1.6	4.4	2.0	5.5
Pancreas	7.1	1.3	6.6	4.4	1.5	5.5
Heart	1.07	0.4		0.8	0.2	

Activities are in counts per minute per gram.

particles were easily thrown out of solution by centrifuging under an acceleration of about 2,000 grams. A sol of this type will be referred to as a large particle sol. Under alkaline conditions (pH 8 to 10) the sols had a characteristically different appearance. They were both red by transmitted light and exhibited little or no blush. The Tyndall beam was faint but was highly polarized. Under dark-field illumination the particles showed a violent Brownian motion and could not be removed from suspension by centrifuging. This sol will be called the small particle sol. Dark-field comparison of samples of the two sols having equal concentrations of gold showed that the small particle sol contained by far the greatest average number of particles per unit volume. Electron microscope pictures of the particles in both of these sols were obtained. These pictures showed that the particles were uniform in size and roughly spheroidal in shape. The large particles were about 200 mμ in size and the small ones about one-fifth to one-tenth as large.

In therapy larger amounts of gold were dispersed at one time. Here the close control of particle size was not attained, but by adjusting the pH rough control was still possible. The therapeutic sols were dispersed under alkaline conditions and contained particles which were on the whole similar to those of the small particle sols. The color to transmitted light was initially reddish violet similar to that of blackberry juice. As time proceeded the color changed to a deeper red. Dark-field studies indicated a greater spread in particle size but a predominance of small particles. It was repeatedly verified that gold sols can be autoclaved at will. Once dispersed they maintain full stability under widely varying conditions of temperature, pH, and salt content.

ADMINISTRATION

Sols were given to the patients via the cubital vein. In most instances an isotonic saline infusion was first begun. The material was added to the infusion and washed in with further saline. In a few cases the sols were given directly by syringe. In one case serial blood samples were taken immediately following injection and at short intervals for about one hour thereafter. The disappearance of gold from the circulation is shown in Figs. 2 and 3. The pertinent clinical data for the patients studied are shown in Table VI. The number of milligrams of material given and the intervals between the last dose and autopsy are included.

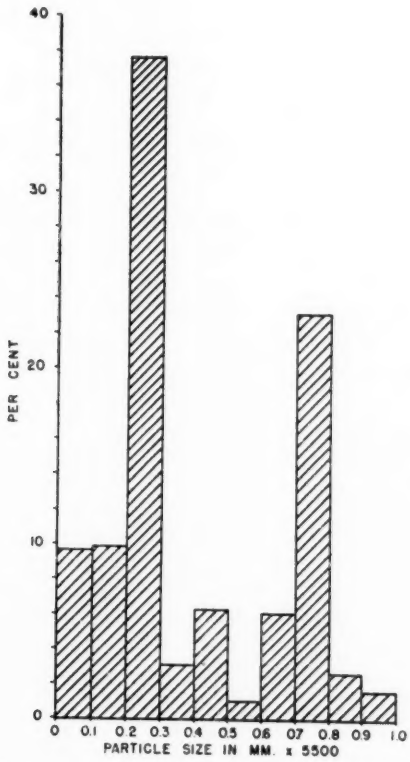

Fig. 1.—Distribution in particle size of a typical manganese dioxide sol.

The dogs were injected via the jugular vein. In all cases female mongrel animals were used. The animals were untrained and were not in a controlled state of nutrition. The animal weights, duration of life after injection, and type of sol given in each case are given in Table VII. In the case of gold each animal (Dogs 1 to 4) received the same total amount of gold, namely, 4 milligrams. A disappearance curve for one dog who received a gold sol of the large particle type is shown in Figs. 4 and 5.

TABLE VI. CLINICAL DATA, DOSAGES, AND INTERVALS BETWEEN LAST DOSE AND AUTOPSY

PATIENT	AGE	SEX	LAST DOSE IN MILLIGRAMS	TIME IN DAYS FROM LAST INJECTION OF ISOTOPE TO AUTOPSY	CLINICAL FEATURES	AUTOPSY
1	41	F	0.7* Mn 0.75 Mn	30 and 50*	Hodgkin's disease, splenomegaly; hepatomegaly, poor liver function, esophageal varices with hemorrhage	Esophageal varices with rupture, cirrhosis of liver; *no* Hodgkin's tissue found
2	8	M	3.0 Mn	4	Typical subacute lymphogenous leucemia	No leucemic infiltration found; bone marrow aplastic; bronchopneumonia; biliary cirrhosis
3	36	M	0.5 Mn	3½	Hypertension with rapidly progressive vascular damage; renal failure	Malignant nephrosclerosis
4	82	M	0.5 Mn	1¼	Arteriosclerosis; chronic congestive heart failure (colored patient)	Cardiac hypertrophy and dilatation; pulmonary congestion; passive congestion of liver and spleen with fibrosis
5	54	M	0.3 Mn	30	Chronic lymphogenous leucemia	Chronic lymphogenous leucemia, with extensive infiltration
6	67	M	0.5 Mn	3 hr. (intraperitoneal)	Carcinoma of the rectum	Adenocarcinoma of rectum; perforation of large bowel; purulent generalized peritonitis
7	7	F	0.6 Mn	20	Acute leucemia	Aplastic bone marrow; very little evidence of leucemia infiltration
8	13	M	1.7 Mn	20	Acute leucemia	Aplastic bone marrow; no evidence of leucemia infiltration
9	10	F	3.8 Mn	1	Acute leucemia	Lymphogenous leucemia with extensive infiltration
10	45	M	0.9 Mn	20†	Carcinoma of cecum with metastases to liver	Adenocarcinoma of cecum; carcinomatosis; aplastic bone marrow
11	10	M	3.5 Mn	2	Acute leucemia	Lymphogenous leucemia with minimal infiltration; aplasia of bone marrow
12	7	M	-‡	13	Acute leucemia	Lymphogenous leucemia with extensive infiltration
13	45	M	-‡	28	Chronic myelogenous leucemia	Leucemia, probably myelogenous, with extensive infiltration
14	57	M	1.8 Au	8	Lymphoblastic lymphosarcoma	Extensive lymphosarcomatous infiltration, primary in retroperitoneal nodes and involving myocardium and abdominal viscera
15	51	M	1.5 Au	12 hr.	Hodgkin's disease	Granulocytopenia, acute; bilateral bronchopneumonia

MnO_2 dosages are expressed as milligrams of metallic manganese.

*Two doses of roughly equivalent effect on tissue activity at autopsy.

†Several doses of roughly equivalent effect on tissue activity at autopsy all greater than twenty days.

‡Patients received both Mn^{52} and Au^{198}; only Mn^{52} effective at autopsy.

RADIOACTIVE ISOTOPES AND MEASUREMENTS OF RADIOACTIVITY

The radioactive manganese* contained principally Mn^{52} of half-life 6.5 days. Small amounts of Mn^{54} were present. Since this isotope has a 310-day half-life, distribution data could be obtained at considerable time after the last dose of manganese. The radioactive gold† was the 2.7-day isotope Au^{198}. No long-lived activity was found after more than ten half-lives of decay.

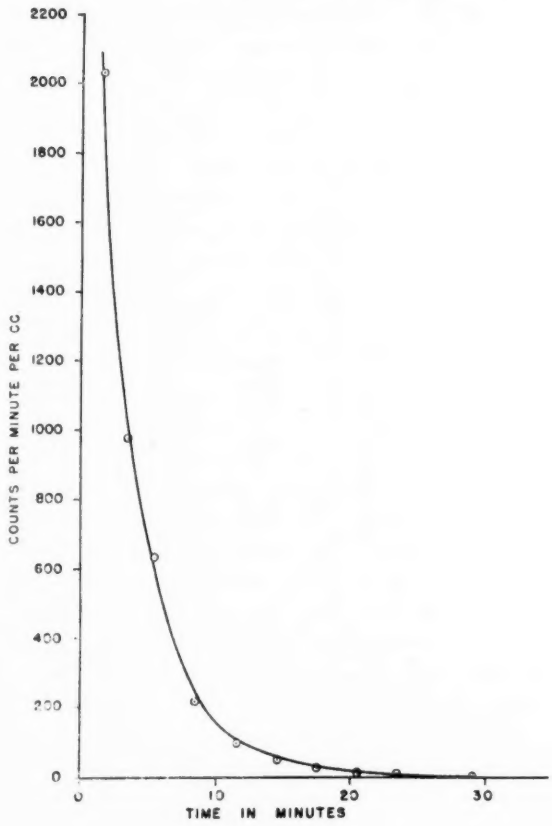

Fig. 2.

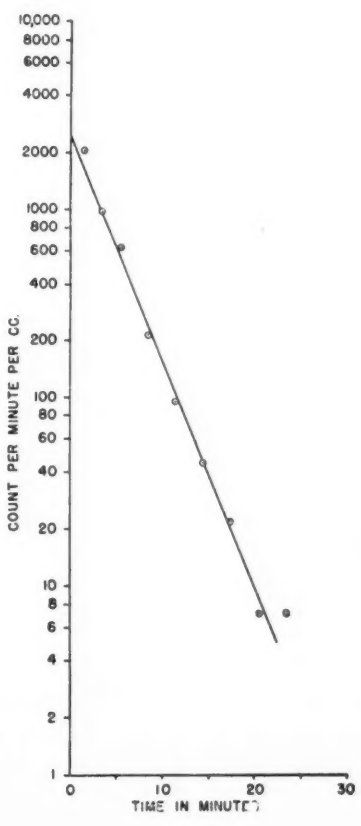

Fig. 3.

Fig. 2.—Disappearance of a therapeutic gold sol from the circulation of a human patient. (Time is measured from the end of the injection period.)

Fig. 3.—Semilogarithmic plot of the data shown in Fig. 2.

At autopsy fresh tissue samples were taken, weighed, dried overnight at 105° C. and then dry ashed in a muffle furnace at 600 to 700° centigrade. A second ashing was frequently required in order to reduce the level of carbonaceous material to a negligible amount in the sample. In the case of manganese dioxide the ash was dissolved in HCl, hydrogen peroxide was added to reduce the manganese, and then each sample was transferred to a small metal

*The radioactive manganese was furnished by the M.I.T. radioactivity center.

†The radioactive gold was obtained from the Manhattan District, Clinton Laboratories, Oak Ridge, Tenn.

ointment tin[4] and dried. In some cases carrier manganese was added; however, no significant difference was found between samples in which this addition was made and others. In a limited number of cases check determinations of the gamma ray activity of samples were made before and after transfer from the beakers in which the ashing was conducted. Transfer was found to be complete, and negligible quantities were left behind. In the case of radioactive gold, nonradioactive gold chloride was added to the samples as a carrier in all cases. After ashing, the gold was taken into solution by adding a few drops of aqua regia. Excess acid was removed by heating before transfer was made to the cups. In the studies of disappearance of radioactivity from the circulation, the blood samples were dried directly in the cups and counted.

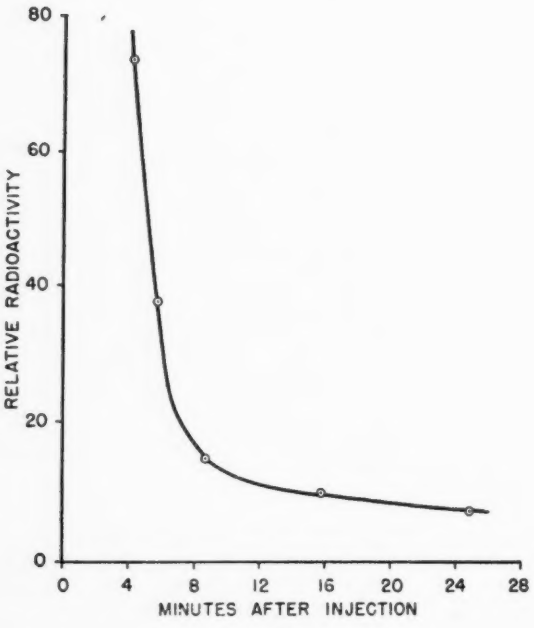

Fig. 4.

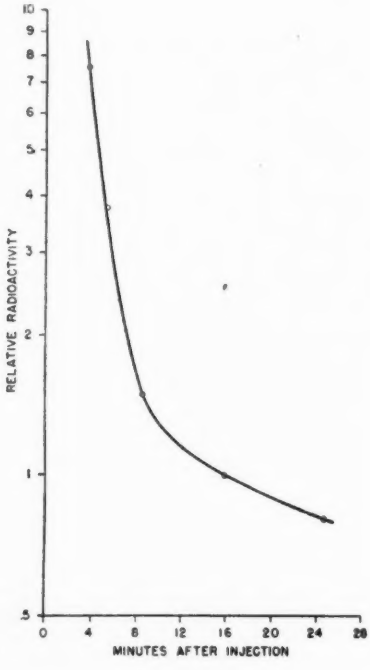

Fig. 5.

Fig. 4.—Disappearance of a large particle gold sol from the circulation of a dog. (Time is measured from the end of the injection period.)

Fig. 5.—Semilogarithmic plot of the data shown in Fig. 4.

Radioactivity measurements were made with the usual thin mica window bell-type Geiger-Müller counter. In the majority of cases the counting tube was connected to a counting-rate meter of the M.I.T. type. In other instances a scaling circuit was employed. Radioactivity was calculated in arbitrary counts per minute per gram of fresh tissue. By determining the activities of tissues at considerable time after autopsy, manganese could be traced in patients who received both elements, since the short-lived gold had then decayed to a negligible level, leaving only the 310-day isotope of manganese

TABLE VII. ANIMAL WEIGHTS, DURATION OF LIFE, AND TYPE OF SOL GIVEN

DOG	WEIGHT (KILOGRAMS)	DURATION OF LIFE AFTER INJECTION	TYPE OF SOL GIVEN
1	8.6	30 min.	Large particle gold sol
2	11.0	30 min.	Small particle gold sol
3	8.0 (Est.)	24 hr.	Large particle gold sol
4	8.0	24 hr.	Small particle gold sol
5	8.0	24 hr.	Manganese dioxide sol
6	9.0	24 hr.	Manganese dioxide sol
7	8.4	48 hr.	Crystalloidal $MnCl_2$ solution

DISCUSSION

Drinker and Shaw[5] injected finely divided manganese dioxide intravenously into cats. Their dispersions were prepared by centrifugation and were probably not of uniform particle size. The particles were less than 1μ in size and were thus small enough to traverse the capillary circulation. The sols were stabilized by adding gum acacia. The disappearance of particles from the circulation was rapid, and in the majority of cases manganese was not found in the blood eighteen minutes after injection. At autopsy 47 per cent of the injected manganese was found in the lung. The content of the liver was 38.3 per cent, of the spleen 4.3 per cent, and of all other organs 10 per cent. Histologic studies indicated that the particles were located within or upon the lining of the capillary endothelium, suggesting that they were not strained out by the capillary bed of the lung by simple filtration. In a separate study Lund, Shaw, and Drinker[6] showed that the cat was unique in this behavior and that rabbits, dogs, guinea pigs, rats, chickens, and turtles concentrated the material predominantly in the liver alone. The content of the spleen was always of the order of 1 or 2 per cent of the initial dose. No separate investigation of the pancreas was conducted.

Since these results suggest that species differences can occur, one could not predict human distribution results with any confidence using our different type of sol prior to the present investigation. The data in Table I are obtained only from single samples of the organs. Only pathologic cases have been studied and absolute percentages of the initial injected dose of manganese in each organ have not been obtained for obvious technical reasons. Nevertheless, the results are similar to those of the previous investigators who use laboratory animals. Thus, we can state with some confidence that the distribution of colloidal manganese in human beings is essentially the same, even when smaller particles are used or smaller amounts of manganese. We hesitate to attach any significance to the lower percentage found in the livers of the two dogs compared to those of the earlier workers, since in our method of preparation an appreciable nondispersed crystalloidal fraction may have been present. Previous experiments have indicated that this is rapidly excreted.[2] The relative amounts are in good agreement with the earlier work.

The high concentration of radioactivity in the pancreas is of great interest. Examination of the data presented by Reiman and Minot[7] shows that the normal human pancreas contains a relatively high concentration of manganese, being

TABLE VIII. DISTRIBUTION OF CRYSTALLOIDAL MANGANESE IN ONE ANIMAL (DOG 7)

ORGAN	COUNTS PER MINUTE PER GRAM	RATIO OF CONCENTRATION TO HEART	PER CENT DOSE
Pancreas	120	7.5	0.02
Liver	180	11.0	1.50
Spleen	19	1.2	0.005
Heart	16	1.0	-
Gall bladder bile	270	17.0	-

exceeded only by the liver. Since the pancreas does not concentrate colloidal gold it is suspected that the manganese in the pancreas was derived, at least in part, from ionic manganese. In a single exploratory experiment an injection of ionic manganous chloride tagged with Mn^{54} was given to a dog. The distribution of tagged manganese in liver (multiple samples), spleen and pancreas (entire organs), and heart is shown in Table VIII. The gall bladder contents were rich in tagged manganese as might be expected from the known biliary excretion of the crystalloidal element. Although this experiment does not prove that all of the manganese in the pancreas was derived from ionic manganese, the high pancreatic concentration is suggestive. A complete understanding of the pancreatic concentration must await experiments with dialyzed colloids and further studies of the metabolism of ionic manganese. It is possible that manganese may play a more significant role in pancreatic metabolism than previously has been realized.

It was noted in Table I that the patients lived for variable intervals after the last dose. This dose in nearly all cases contributed almost entirely to the radioactivity at autopsy. There appears to be no really significant change in relative concentration of radioactivity as the elapsed time following the last injection increases. Nevertheless, the values are subject to rather large variations which may obscure small effects.

The doses which were administered were always small. In the case of gold less than 3 mg. were given to a typical patient. In the case of manganese the maximum dose was 3.8 mg. of metallic manganese equivalent (Patient 9), and the majority of doses varied between 0.3 and 1 milligram. Here again the data do not give any picture of variation of distribution with the size of the dose.

It is shown in Fig. 2 that colloidal gold disappears rapidly from the human circulation and that under proper conditions the semilogarithmic plot (Fig. 3) is strictly linear for a considerable period of time. The appreciable time for injection (two or three minutes) plus the short interval before the first blood sample was obtained (one and one-half minutes) is evidently ample to insure circulatory mixing. The exponential decay with a "half-life" of two and one-half minutes suggests that the rate of phagocytosis is strictly proportional to the concentration of colloidal particles in circulation. The disappearance curve for the dog (Figs. 4 and 5) indicates a correspondingly rapid decline. The nonlinearity of the disappearance curve suggests that the dog was not in a uniform circulatory state. Since the animal was not trained and struggled frequently during the experiment, this was not surprising.

The distribution of colloidal gold in different samples of liver and spleen in Dog 4 indicates a systematic variation (Table IV). The activity of equivalent central samples of each lobe is essentially the same, but a systematic increase in concentration is indicated between the peripheral and central portion of the right central lobe in which multiple samples were taken. Similarly, there was a significant variation in the concentration of gold in the spleen samples. Bearing this in mind, it is not difficult to understand the definite variations in comparative results in all cases where determinations were made on single samples of tissue. In spite of such variations, however, the picture is consistent in that liver and spleen are roughly equivalent in concentration of radioactivity and outstandingly high compared to all other tissues. This distribution is very similar to that found by Jones, Wrobel, and Lyons[8] in a single dog which received an intravenous injection of colloidal chromic phosphate containing particles 1 μ or less in size.

The different splenic concentrations for gold and manganese is noteworthy indeed. If we are concerned with true phagocytosis, there must be a characteristic difference between the phagocytic action of the spleen for different chemical substances, such that the affinity of the spleen for manganese dioxide is relatively less than for gold. It is of interest that Voigt[9] observed a relatively lower concentration of colloidal silver in the spleen of the rabbit.

Although only one intraperitoneal injection was given, it is of considerable interest that the distribution is roughly similar to that obtained by intravenous injection. Additional research is required before definite conclusions can be established in this matter.

SUMMARY

A study of the relative distribution of colloidal particles of manganese dioxide containing radioactive Mn^{52} has shown that in the human being liver concentration is high. The concentration of the pancreas and kidney is intermediate and that of the spleen and other organs is low. Two dogs showed a similar picture for liver, pancreas, spleen, and heart, which were the only organs studied. Studies in human subjects and dogs of the distribution of colloidal gold containing radioactive Au^{198} showed a high concentration in liver and spleen, intermediate to low in the kidney, and a low concentration in other organs. The difference in the splenic concentration of gold and manganese is striking and suggests a fundamental difference of phagocytic activity for the two substances. The high pancreatic concentration of manganese is of especial significance.

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EFFECT OF PREGNANCY ON THE COMPLEMENT OF GUINEA PIGS

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ALTHOUGH it is generally known among serologists that the serum of a pregnant guinea pig is likely to be unsatisfactory for use in complement fixation, no study of the exact relationship between pregnancy and complement in this animal has been found in the literature. The question is of importance as a preliminary step in the study of complement variations in disease, since some knowledge of the possible variations of complement in different physiologic states is necessary for the proper interpretation of data obtained from a group of experimental animals. The present study was undertaken in an attempt to delineate the range of variation of complement in pregnant guinea pigs.

METHODS

Selection and Care of the Animals.—A group of nine healthy adult guinea pigs, known to be nonpregnant, was selected as a control in the experiment. Members of the group ranged in age from 10 to 14 months and in weight from 450 to 700 grams.

The group of pregnant guinea pigs was composed of females recently bred or known to be pregnant. The group was screened to exclude any animals that did not correspond approximately to the control group in respect to age, size, and general health. Various stages of pregnancy were represented in the group, particularly the later stages. A total of fourteen animals was observed both during and after parturition. Two additional animals were acquired after they had given birth and were observed only during the postparturition period.

All animals were maintained separately in clean, dry cages. They were fed a controlled diet of oats, Rockland guinea pig Pellets, dried alfalfa, and fresh cabbage. No water was offered to the animals at any time.

The time of parturition of each pregnant guinea pig was recorded from actual observation or from an estimate based on the appearance of the litter after birth had occurred.

Method of Obtaining Blood Samples.—A blood sample of 0.4 to 0.6 c.c. was withdrawn twice weekly from each guinea pig by bleeding from a small incision in the marginal vein of the ear. This method appears to have no undesirable effects upon the animal and, for that reason, is to be preferred to the more common method of cardiac puncture, particularly in a study of this type.

The blood sample was collected in a clean 3 c.c. serologic test tube, covered with a small piece of parafilm to protect the sample and retard evaporation,

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allowed to clot for twenty minutes at 2 to 4° C., and then centrifuged at 1,600 r.p.m. for fifteen minutes. The supernatant serum was decanted carefully into another clean 3 c.c. test tube and stored at 2 to 4° C. until used. In all cases the serum was titrated within two hours after collection of the sample.

Method of Titration.—The assay of complement was performed by a method independently devised in this laboratory. Similar methods have been used by Mayer, Eaton, and Heidelberger[1] and by Kent, Bukantz, and Rein.[2]

The serum to be tested was diluted 1:100 in 0.9 per cent saline solution. Immediately after dilution, portions of the diluted serum were pipetted into each of three clear glass test tubes (of the type designed for use in the Klett-Summerson photoelectric colorimeter). For sera of a high titer, the portions used were 1.00, 1.50, and 2.00 c.c., respectively. For sera of low titer, portions of 1.50, 2.00, and 2.50 c.c. were used. Varying the quantities in this fashion provided an optimal amount of serum in at least two of the three tubes. The volume in each tube was adjusted to 2.50 c.c. by the addition of saline solution.

To each tube 2.50 c.c. of a suspension of sensitized sheep red cells (prepared as herein described) were then added. The tubes were shaken thoroughly and incubated for thirty minutes at 37° Centigrade. After the incubation period, the tubes were centrifuged at 1,600 r.p.m. for five minutes.

The color of the supernatant fluid in each tube was measured with a Klett-Summerson photoelectric colorimeter equipped with a standard green filter over the light source. The scale of the instrument is calibrated in units which are proportional to the concentration of dissolved hemoglobin in the supernatant layer. Thus, the per cent hemolysis occurring in a given tube may be computed as the ratio of the color reading to that of the supernatant layer in a completely hemolyzed sample. The latter value was determined for each set of titrations by taking the color reading of 2.50 c.c. of the sensitized cell suspension laked in 2.50 c.c. of distilled water.

Preparation of the Standard Sheep Cell Suspension.—One volume of freshly drawn packed sheep erythrocytes was washed four times in 10 volumes of saline solution. The washed, packed cells were suspended in saline solution in sufficient amount so that a sample of this suspension (diluted 1:20 in saline solution) gave a color reading of 280 on the colorimeter. When thus adjusted in concentration, the cell suspension was then diluted with an equal volume of saline solution containing 5 units of rabbit antisheep hemolysin of a titer of at least 1:4,000. After mixture of the cells and hemolysin, the suspension was allowed to stand for thirty minutes at 2 to 4° C. before use. The final suspension contained about 2 per cent (by volume) of packed cells and approximately 2.5×10^8 erythrocytes per cubic centimeter.

Calculation of Results.—The amount of 1:100 serum required to effect 50 per cent hemolysis was obtained from a form of the von Krogh equation (3):

$$x = k \left(\frac{y}{1 - y} \right)^{1/n}$$

Where x equals amount of hemolytic serum, y equals fraction of total cells hemolyzed, and k and 1/n are constants for a given set of experimental conditions. When expressed in the logarithmic form and adapted to the case of 50 per cent hemolysis, the equation may be written as:

$$x_2 = \text{antilog}\left(\log x_1 - \frac{1}{n} \log \frac{Y}{100 - Y} \right)$$

Where x_2 equals cubic centimeters of 1:100 serum required for 50 per cent hemolysis, x_1 equals cubic centimeters of 1:100 serum actually used, and Y equals per cent hemolysis actually obtained. For the titration used, the value of the constant 1/n was found to be 0.22 ± .02. Curves of x_2 versus $\frac{Y}{100 - Y}$ were plotted on log-log graph paper for the various discrete values of x_1. From the resulting straight-line nomograph, the value of x_2 for any values of x_1 and Y could be determined by interpolation.

The von Krogh equation is a satisfactory empirical statement of the relationship only when the hemolysis is between 10 and 90 per cent. The serum portions (x_1) have been chosen so that the resulting hemolysis will ordinarily be within this range. In this experiment the value of x_2 was taken as the average found in the tubes (usually all three) in which more than 10 but less than 90 per cent hemolysis occurred.

The *complement activity* was defined arbitrarily as the number of 50 per cent hemolytic units per cubic centimeter of serum. In terms of x_2 this is expressed as:

$$\text{Complement activity} = \frac{100}{x_2}$$

Complement activity bears a direct relationship to the concentration of complement in the serum and provides a convenient figure for graphing and tabulation.

RESULTS AND DISCUSSION

The results of 262 separate complement titrations are shown as a "scatter diagram" in Fig. 1. Complement activity is depicted as a function of time in days relative to the date of parturition. For the nonpregnant guinea pigs, the distribution of points along the time axis was in terms of the number of days before or after the middle of the observation period.

Inspection of the graph apparently discloses that (1) the pregnant guinea pigs had substantially lower complement activities than the nonpregnant ones and (2) the postparturition period in the group of pregnant guinea pigs was marked by a gradual increase in complement activity.

These observations may be further substantiated by statistical analysis.* The average complement activity for each nonpregnant guinea pig is shown

*The authors are indebted to Dr. Huldah Bancroft, Department of Preventive Medicine, Medical School, Western Reserve University, for assistance in the statistical evaluation.

TABLE I. COMPLEMENT ACTIVITY IN NONPREGNANT GUINEA PIGS

	GUINEA PIG									GROUP MEAN
	1	9	10	24	35	36	44	45	46	
Days in observation period	37	37	45	42	37	37	16	16	16	--
Number of separate titrations	11	11	14	13	10	11	5	5	5	--
Average C′ activity	80	84	92	67	59	84	74	62	68	74
Highest C′ activity	100	122	126	86	80	110	84	82	87	--
Lowest C′ activity	52	55	67	55	44	61	59	47	49	--

in Table I; the average complement activities for the pregnant animals, both before and after parturition, are shown in Table II. The animals were divided into three groups: nonpregnant, ante partum, and post partum. The statistical significance of the differences among the means of complement activity in the three groups was determined by the method devised by Fisher[4] for groups small in number.

The results of the statistical analysis are shown in Table III. The value of "t" in the tests of significance are well above the minimum values required to demonstrate significant differences among the three groups.

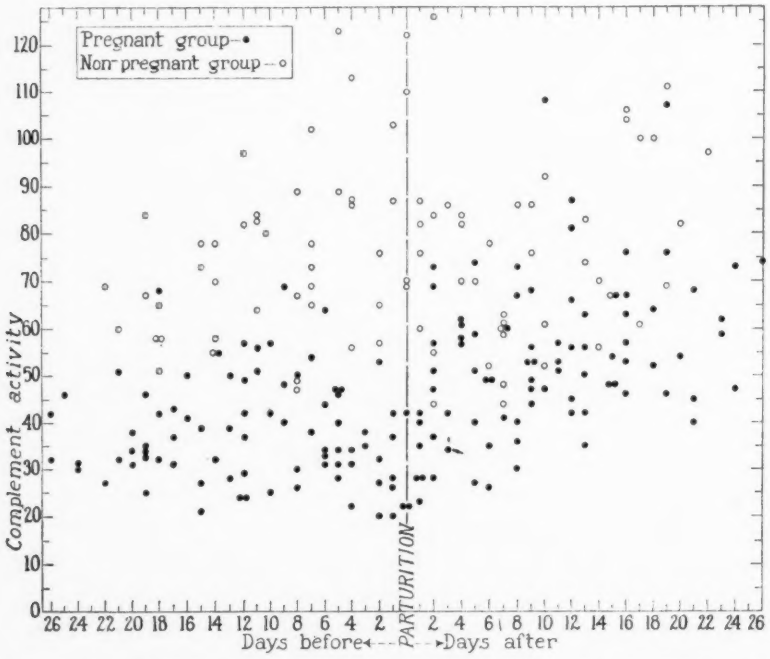

Fig. 1.—Scatter diagram of complement activity before and after parturition. Points for the nonpregnant group are located according to the number of days before or after the middle day of the observation period.

It should be pointed out that, although a significant difference in complement activity between the nonpregnant and post-partum groups exists, the gradual upward trend in the post-partum group predicts that most of these animals would have returned to normal within five weeks after parturition. This prediction is confirmed by the frequent observation of the authors that the serum

TABLE II. COMPLEMENT ACTIVITY IN PREGNANT GUINEA PIGS BEFORE AND AFTER PARTURITION

	GUINEA PIG															GROUP MEAN
	21	25	26	27	28	29	30	31	32	33	34	37	38	41	42	43	
Before Parturition																	
Days in observation period	21	19	7	22	3	31	35	25	19	25	13	20	27	0	0	12	--
Number of separate titrations	7	6	2	7	2	10	11	7	6	7	4	11	8	0	0	3	--
Average C′ activity	43	34	30	31	21	32	47	43	27	36	31	45	49	-	-	33	36
Highest C′ activity	53	51	33	38	22	42	69	64	34	44	47	57	68	-	-	35	--
Lowest C′ activity	37	22	27	25	20	20	32	30	21	22	24	35	28	-	-	29	--
After Parturition																	
Days in observation period	19	16	26	18	30	15	11	12	13	12	24	17	10	25	25	3	--
Number of separate titrations	6	5	8	6	8	5	4	4	5	4	7	5	3	7	7	2	--
Average C′ activity	62	44	67	44	48	54	57	58	57	51	49	57	79	55	40	42	54
Highest C′ activity	76	50	107	61	74	67	67	81	92	66	73	76	108	68	48	42	--
Lowest C′ activity	44	34	37	23	26	47	51	35	28	28	35	40	60	40	27	42	--

TABLE III. STATISTICAL EVALUATION OF DIFFERENCES AMONG NONPREGNANT, ANTE-PARTUM, AND POST-PARTUM GROUPS OF GUINEA PIGS

GROUPS COMPARED	FIRST MEAN ($\bar{x}_1$)	NO. (n_1)	SECOND MEAN ($\bar{x}_2$)	NO. (n_2)	S.D. OF DIFFERENCE (s^2)*	DEGREES OF FREEDOM	t†	MINIMUM VALUE OF t FOR P = 0.01
Nonpregnant group and Ante-partum group	74	9	36	14	90.2	21	9.4	2.831
Ante-partum group and Post-partum group	36	14	54	16	82.0	28	6.2	2.763
Nonpregnant group and Post-partum group	74	9	54	16	106	23	4.7	2.797

*Standard deviation of difference $= s^2 = \frac{\Sigma (x_1 - \bar{x}_1)^2 + \Sigma (x_2 - \bar{x}_2)^2}{n_1 + n_2 - 2}$

†$t = \frac{|\bar{x}_1 - \bar{x}_2|}{s} \sqrt{\frac{n_1 n_2}{n_1 + n_2}}$

of a female guinea pig four to six weeks after parturition is fully as active in fixation and hemolysis as the sera of adult virgin female guinea pigs kept under the same dietary conditions.

CONCLUSIONS

1. Pregnancy in the guinea pig is accompanied by a substantial decrease in complement activity of the serum.

2. After parturition complement activity gradually increases toward the level normal for nonpregnant female guinea pigs.

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TOXICITY STUDIES OF α (4-HYDROXY-3,5-DIIODO-BENZYL-N-BUTYRIC ACID) AND α (4-HYDROXY-3,5-DIIODO-BENZYL-N-CAPROIC ACID): COMPOUNDS PROPOSED AS GALL BLADDER CONTRAST AGENTS

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VISUALIZATION of the gall bladder through the use of a new series of compounds has been described in a recent report.* Of these α (4-hydroxy-3,5-diiodo-benzyl-n-butyric acid) and α (4-hydroxy-3,5-diiodo-benzyl-n-caproic acid) were selected as having superior properties from the point of view of concentration in the gall bladder with a minimum of reaction of the patient. Since these compounds may be used extensively in clinical work it was deemed advisable to undertake a study of their toxicity.

Both substances appear as fine, white crystalline powders. They are insoluble in water but soluble as the sodium salts. Through the use of sodium hydroxide α (4-hydroxy-3,5-diiodo-benzyl-n-butyric acid) forms a solution with a pH of 7.5, while under similar conditions α (4-hydroxy-3,5-diiodo-benzyl-n-caproic acid) forms a solution of pH 8.5. The compounds were purified before use by dissolving them in 5 per cent $NaHCO_3$ solution (1 Gm. to 100 c.c. of bicarbonate solution). Charcoal was added and the solution filtered. The acids, precipitated by the addition of concentrated hydrochloric acid, were filtered and, after drying, recrystallized from carbon tetrachloride.

EXPERIMENTAL PROCEDURE

For intravenous administration a 25 per cent solution of each compound was used. For oral use the powder was intimately mixed with small amounts of ground red meat or fish. In all experiments cats of 2.5 to 4 kilograms of body weight were used. These cats were observed for a period of one week before starting the experiments.

During the experiment the weights of each animal were recorded frequently and their general behavior and reaction to the drugs were observed. The cats were then sacrificed at the termination of the period of observation, which varied from two to six weeks. Necropsy studies then were made.

DISCUSSION OF RESULTS

Administration of these drugs to cats by the oral route causes much less evidence of toxic effects than is the case with intravenous administration. With the exception of an impaired appetite for a period of forty-eight hours, the cats showed no untoward reaction to the oral administration of the drugs. Vomiting was not observed at any time and all the animals gained weight. During the period of observation two of the cats delivered normal litters of kittens. At

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TABLE I. EFFECT OF ORAL ADMINISTRATION OF SINGLE DOSE OF α (4-HYDROXY-3,5-DIIODO-BENZYL-N-BUTYRIC ACID)

CAT	DOSE/KG. (MG.)	TOTAL DOSE (GM.)	PERIOD OF OBSERVATION (WEEKS)	REMARKS
22	750	1.60	4	No vomiting; appetite impaired for 48 hours
1	910	3.19	6	No vomiting; appetite impaired for 48 hours
6	940	3.20	6	No vomiting; appetite impaired for 48 hours
2	950	4.09	6	No vomiting; appetite impaired for 48 hours
25	1,000	3.20	4	No vomiting; appetite impaired for 48 hours
18	1,000	2.60	3	No vomiting; appetite impaired for 48 hours
19	1,000	3.10	3	No vomiting; appetite impaired for 48 hours
27	1,000	1.90	3	No vomiting; appetite impaired for 48 hours
26	1,000	1.40	3	No vomiting; appetite impaired for 48 hours
24	1,000	3.50	3	No vomiting; appetite impaired for 48 hours
31	1,000	3.70	3	No vomiting; appetite impaired for 48 hours

TABLE II. EFFECT OF ORAL ADMINISTRATION OF SINGLE DOSE OF α (4-HYDROXY-3,5-DIIODO-BENZYL-N-CAPROIC ACID)

CAT	DOSE/KG. (MG.)	TOTAL DOSE (GM.)	PERIOD OF OBSERVATION (WEEKS)	REMARKS
20	830	3.07	4	No vomiting; appetite impaired for 48 hours
7	835	3.09	6	No vomiting; appetite impaired for 48 hours
30	840	3.02	4	No vomiting; appetite impaired for 48 hours
3	845	2.45	6	No vomiting; appetite impaired for 48 hours
21	885	3.01	4	No vomiting; appetite impaired for 48 hours
5	890	3.20	6	No vomiting; appetite impaired for 48 hours
4	910	3.19	6	No vomiting; appetite impaired for 48 hours
23	1,000	2.50	4	No vomiting; appetite impaired for 48 hours
29	1,000	3.10	3	No vomiting; appetite impaired for 48 hours
28	1,000	2.70	3	No vomiting; appetite impaired for 48 hours

necropsy there were no abnormal findings either in the gross or in histologic sections, except that a minimal degree of cloudy swelling was noted in the liver and kidneys of two cats.

No estimation was made of the lethal dose orally, because all animals survived doses of 1,000 mg. per kilogram of body weight. Higher doses were not studied, for the total dose administered to human beings orally does not exceed 100 mg. per kilogram of body weight.

TABLE III. EFFECT OF INTRAVENOUS ADMINISTRATION OF SINGLE DOSE OF α (4-HYDROXY-3,5-DIIODO-BENZYL-N-BUTYRIC ACID) TO UNANESTHETIZED CATS

CAT	DOSE/KG. (MG.)	TOTAL DOSE (MG.)	PERIOD OF OBSERVATION (WEEKS)	REMARKS
2a	175	810	6	No untoward reaction
27a	175	330	6	No untoward reaction
3a	180	540	6	Salivated; slight ataxia completely recovered in 30 minutes
4a	260	1,090	6	No untoward reaction
28a	300	810	6	No untoward reaction
5a	310	1,080	6	Drowsy; occasional jerky movements; deep respirations
1a	350	1,240	6	Salivated; no other reaction
19a	350	1,085	6	Rapid respirations; recovered in 1 hour
7a	380	1,350	6	Salivated; occasional jerky movements; recovered in 45 minutes
18a	400	1,040	6	Rapid respirations; coma; recovered after several hours
30a	500	1,850	-	Rapid breathing; coma; death after 45 minutes

TABLE IV. EFFECT OF INTRAVENOUS ADMINISTRATION OF SINGLE DOSE OF α (4-HYDROXY-3,5-DIIODO-BENZYL-N-CAPROIC ACID) TO UNANESTHETIZED CATS

CAT	DOSE/KG. (MG.)	TOTAL DOSE (MG.)	PERIOD OF OBSERVATION (WEEKS)	REMARKS
14a	227	750	6	Rapid respirations; coma; twitchings; fully recovered in 24 hours
13a	240	750	6	Rapid respirations; coma; twitchings; fully recovered in 24 hours
9a	275	1,275	6	No reaction; took feeding 1 hour later
16a	275	625	4	Rapid breathing; twitchings during 30 minutes
12a	290	875	4	Mild convulsions; rapid breathing; recovered in 1 hour
8a	305	1,325	6	Convulsions; rapid breathing; coma; recovered in 24 hours
10a	315	1,025	4	Convulsions lasting for about 1 hour; coma; recovered after 24 hours
11a	335	1,250	6	Rapid respirations; coma; twitchings; fully recovered in 24 hours
15a	375	750	-	Violent convulsions; coma; died after 15 minutes

Study of α (4-hydroxy-3,5-diiodo-benzyl-n-butyric acid), in intravenous doses below 200 mg. per kilogram, failed to demonstrate any abnormal reactions. Above this level the administration of the drug caused convulsions, rapid respiration, and varying degrees of drowsiness; such reactions were more marked above 300 mg. per kilogram. When 400 mg. per kilogram were administered intravenously the cats became comatose, and at 500 mg. per kilogram death followed after a coma of forty-five minutes' duration. The lethal intravenous dose of this drug is of the order of 400 to 500 mg. per kilogram.

Following the intravenous studies of α (4-hydroxy-3,5-diiodo-benzyl-n-butyric acid), the intravenous studies of α (hydroxy-3,5-diiodo-benzyl-n-caproic acid)

were started at a level exceeding 200 mg. per kilogram of body weight. At levels of 200 to 275 mg. per kilogram rapid respiration was noted, together with some ataxia and twitchings of the extremities. At 300 mg. per kilogram convulsions were observed, and at 315 mg. per kilogram the convulsions were marked, with rapid breathing. This rapid breathing persisted for several hours. When the dose was raised to 375 mg. per kilogram death of the injected animal occurred after fifteen minutes.

The animals that survived the intravenous administration of the drugs recovered completely after twenty-four to forty-eight hours. They took their feeding well thereafter and gained weight. When the animals were sacrificed at the end of two to four weeks no abnormal findings were observed on gross examination of the liver and kidneys.

In the two animals that died following the administration of the lethal doses, petechial hemorrhages were noted in the lungs. The livers and kidneys showed moderate degrees of cloudy swelling.

SUMMARY

1. The toxic effects of α (4-hydroxy-3,5-diiodo-benzyl-n-butyric acid) and α (4-hydroxy-3,5-diiodo-benzyl-n-caproic acid) were studied, both by oral and by intravenous administration, in a total of forty-two cats.

2. Oral administrations of the drug were tolerated in doses up to 1,000 mg. per kilogram, without vomiting. Appetite was impaired for forty-eight hours.

3. The intravenous administration of α (4-hydroxy-3,5-diiodo-benzyl-n-butyric acid) caused convulsions in doses higher than 200 mg. per kilogram and death at 500 mg. per kilogram. Intravenous administration of α (4-hydroxy-3,5-diiodo-benzyl-n-caproic acid) caused convulsions at 200 mg. per kilogram and death at 375 mg. per kilogram.

4. All the animals given sublethal doses of the drugs recovered completely and gained weight at the end of two to four weeks.

5. Necropsy studies revealed no abnormal findings, except in cats which were given lethal doses of the drugs. In these cats petechiae in the lungs and cloudy swelling in the liver and kidneys were found.

LABORATORY METHODS

THE MICROCOLORIMETRIC ESTIMATION OF PLASMA PROTEINS

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IN A recent publication[1] we described a colorimetric method utilizing the Sakaguchi reaction for the estimation of total proteins and protein fractions in human plasma, nephrotic urine, and abdominal fluid in terms of arginine which was shown to have an accuracy comparable to the micro-Kjeldahl method. Subsequent experiments have shown that because of the relatively negligible nonprotein arginine content of the blood greater economy of time and blood sample without appreciable loss of accuracy can be achieved by: (1) determination of total plasma proteins on a 1 c.c. aliquot of a sample prepared by diluting 0.1 c.c. of plasma to 2 c.c. volume with 10 per cent NaOH instead of an alkaline solution of the trichloroacetic acid precipitated proteins, (2) estimation of albumin and globulin by direct measurement of both proteins as arginine in fraction derived from the same 0.2 c.c. sample of plasma rather than by difference of total protein and globulin, and (3) elimination of the three-hour incubation period usually employed in the separation of albumin and globulin fractions of the plasma. The rapidity and ease of operation which has been gained by these modifications would seem to recommend the method for routine clinical work.

EXPERIMENTAL

Reagents.—

Sodium Hypochlorite: A 0.06 N solution was prepared as needed from the commercially available Clorox. The necessary dilution of the stock product was ascertained iodometrically as follows: To 1 c.c. of Clorox in a 125 c.c. Erlenmeyer flask are added 25 c.c. of chlorine-free water in which has been dissolved 1 Gm. of potassium iodide. The mixture was then titrated with 0.1 N sodium thiosulfate, 1 c.c. of starch indicator being used. The stock product has been found to be fairly stable for three to four months if kept in the refrigerator in a brown bottle.

Sodium Hydroxide: A 10 per cent solution.

Urea: A 20 per cent solution.

α-Naphthol: One hundred milligrams of the resublimed product are dissolved in 100 c.c. of 95 per cent ethanol. The solution is kept in a brown bottle and stored in the refrigerator.

Arginine Standard: One (plus) arginine hydrochloride* (26.6 per cent N found), 12.05 mg., is weighed accurately and dissolved in 100 c.c. of a saturated aqueous solution of benzoic acid. One cubic centimeter of this solution is equivalent to 100 γ of the free base. This solution is stored in the refrigerator.

Ether: Reagent grade can be used as obtained.

Sodium Sulfate, Anhydrous: A 22 per cent solution is employed and is stored in a 37° C. incubator.

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Analytic Procedures.—

Total Plasma Proteins: One-tenth cubic centimeter of plasma (derived from blood collected over lithium oxalate) in a graduated 15 c.c. conical centrifuge tube was made to the 2 c.c. mark with 10 per cent NaOH. A 1 c.c. aliquot of the thoroughly mixed sample was transferred to a graduated colorimeter tube, and 5 c.c. of water and 1 c.c. of alcoholic naphthol were added in rapid succession. After standing for five minutes, 1 c.c. of sodium hypochlorite is added, followed exactly one minute later by the addition of 2 c.c. of 20 per cent urea solution. The resulting solutions are mixed by inverting the tubes and read in the Klett-Summerson colorimeter with Filter S-54 after a lapse of five minutes. The color intensity of the test mixture has been found to remain constant for an hour or more after the initial reaction period, so that there is no need to hasten the color measurement. A parallel determination is also done on a 1 c.c. aliquot of the standard and a reagent blank.

Plasma Albumin and Globulin: To 0.2 c.c. of plasma in a 50 c.c. round bottom, small neck centrifuge tube (A. H. Thomas No. 3123) was added with mixing 7 c.c. of 22 per cent sodium sulfate and then 3 c.c. of ethyl ether.[2] This mixture was shaken vigorously for two minutes and then centrifuged at 3,000 r.p.m. for ten minutes. The globulin which collected at the ether-water interface was separated from both liquid layers by careful decantation into a 25 c.c. graduated cylinder. The volume of the aqueous, albumin-containing fraction was adjusted to 10 c.c. by the addition of water, and a 5 c.c. aliquot of this layer is removed by pipette to a 10 c.c. graduated colorimeter tube. Subsequent to the addition of 1 c.c. of 10 per cent NaOH, the protein content is determined in terms of arginine described as follows.

The disk of precipitated globulin was dissolved in 2 c.c. of 10 per cent NaOH and then 2 c.c. of water were added. Gentle heating of the mixture in a hot water bath drives off the residual ether and thereby greatly facilitates solution of the protein. A 2 c.c. aliquot of the cooled sample was transferred to a colorimeter tube and submitted to the Sakaguchi reaction after the addition of 4 c.c. of water.

The alkaline dilution of the whole or fractionated plasma of specimens containing 400 mg. per cent of cholesterol or more should be shaken with 5 c.c. of ether prior to removal of sample for the arginine determination.

Attention is called to the fact that in all revisions of sample size which different circumstances may necessitate, it is imperative that the sample be contained in a 6 c.c. volume which shall include the equivalent of 1 c.c. of 10 per cent NaOH prior to the addition of the other reagents.

Calculations: After the colorimeter is set to zero for the reagent blank, the arginine present in the protein samples is estimated by comparison with the value of the arginine standard. The protein equivalent is obtained by multiplying the arginine values by the dilution factors and 19.2, a factor derived from the mean arginine content of human plasma albumin and globulin.[3]

RESULTS

The possibility of the present modification of our previously described method for the determination of plasma proteins was impressed upon us by the finding that protein-free filtrates obtained from 5 c.c. samples of ten different human plasma gave negative Sakaguchi tests. This observation indicated that failure to separate the proteins from whole plasma would not introduce any appreciable errors in the final values.

Our experiments amply confirmed the observations of Kingsley[2] that when ether is employed in conjunction with sodium sulfate no preliminary incubation period is required for the separation of globulin and albumin. It has also been found that serum or plasma derived by the use of other oxalates or other anticoagulants can be used with this method.

The total plasma values obtained by the usual micro-Kjeldahl method and three variants of the arginine method are listed in Table I. It will be noted

TABLE I. COMPARISON OF TOTAL PLASMA PROTEIN VALUES OBTAINED BY VARIOUS TECHNIQUES

SPECIMENS	MICRO-KJELDAHL METHOD, PROTEIN = N × 6.25 (GM. %)	ARGININE METHOD, PROTEIN = ARGININE × 19.2		
		TRICHLOROACETIC ACID-PRECIPITATED PROTEIN (GM. %)	PLASMA DILUTION (GM. %)	TOTAL PLASMA PROTEIN = ALBUMIN + GLOBULIN (GM. %)
Ka, normal, 2 years	7.65	7.70	7.66	7.73
Ec, nephrotic, 1.5 years	3.80	3.84	3.80	3.93
As, nephrotic, 2 years	5.75	5.74	5.78	5.78
Sa, nephrotic, 3 years	4.48	4.46	4.57	4.58

that the figures obtained by the two techniques described in the present report, dilution of the whole plasma and addition of the individually determined albumin and globulin values, are in good accord with those obtained with the arginine method utilizing the trichloroacetic acid-precipitated plasma proteins as well as those of the micro-Kjeldahl procedure.

COMMENT

The accuracy, speed of operation, and economy of sample achieved by the present modification of the arginine method for the determination of plasma proteins would seem to favor its adoption for routine or clinical studies. Although these procedures are somewhat more involved and time consuming than the specific gravity methods,[4] the higher degree of accuracy which they afford makes the additional effort which they entail seem worth while. This is especially true of the copper sulfate method which has recently been shown by Adams and Ballou[5] to exhibit such poor correlation between the specific gravity measurement and protein content as determined by the micro-Kjeldahl procedure as to make it valueless as a measure of total serum proteins of the blood. This and similar findings reported by Looney[6] and Zozaya,[7] however, have been contradicted by the observations of Atchley and co-workers.[8] The biuret method of Kingsley[2] which is also rapid and employs only small amounts of plasma proved unsatisfactory in our hands. Our difficulties arose from (1) lack of stability of the alkaline copper sulfate reagent, (2) development of uncontrollable turbidity in test samples, especially with specimens from nephrotic patients, and (3) lack of consistent correlation of biuret plasma or serum values with micro-Kjeldahl figures.

Attention is called to the fact that in physiologic or pathologic hyperaminoacedemia, which would naturally tend to increase the amount of free circulating arginine, the plasma or serum proteins should be precipitated with trichloroacetic acid prior to the application of the Sakaguchi reaction as previously reported,[1] rather than employ the procedure described here.

SUMMARY

A rapid microcolorimetric method for the determination of total plasma proteins or plasma albumin and globulin has been described which yields values comparable to those obtained by the micro-Kjeldahl procedure.

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A PULSATING PERFUSION APPARATUS

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THIS apparatus, announced in Science,[2a] is a considerable improvement over the one described in 1939,[1] differing in form and size, embodying some entirely new additions, yet retaining the essential features of construction of the older apparatus. Its general appearance is shown in Fig. 1, and details of construction are shown in Figs. 2 to 6. It is so planned that it can operate on a small quantity of blood and that the organ can be studied under a microscope during perfusion. With the apparatus and its specimen chamber shown in Fig. 1, the stomach, pancreas, and part of the duodenum of the rat can be perfused with whole rat blood.

In Figs. 2 to 6 only the essentials are shown. The chief portion of the apparatus consists of a number of pieces of ⅛ inch plate glass, the largest (Plates I and II) measuring 4⅜ by 7 inches, held together by twelve bolts and milled nuts. The latter are not shown in the drawings given, but in Fig. 5 their positions are indicated. Most of the other essential parts are fabricated from glass tubing and are attached to the plates by ground taper joints.

The channels or "vessels" through which the blood or other fluid circulates consist of small holes drilled through the plates and half-round grooves cut in one plate and covered by another. Most of these grooves are on the under side of the second plate (II) from the bottom; some are in other plates as shown in the drawings. The arrangement or plan of these channels and their relations to the reservoir and pump and other parts are shown in Fig. 5. Some appear in section in Fig. 6.

The essential parts of the pump are two valve chambers, two valves, the three rubber tubes and bulbs, connecting channels, and the electromagnet and mechanism for compressing the middle tube (No. 2). The valve chambers are large holes cut in the middle plate (IV) of the three making up the pump. The valves are small triangular disks of glass (Figs. 3, 5, 6), one of which rests on the openings in the bottom of each chamber. The "cylinder" is tube No. 2, and the "piston" of the pump is the fluid in tube No. 2, which moves up and down as the rubber tube is rhythmically compressed by the magnet and its attached mechanism and released to expand by its own elasticity. The connecting channels (2 and 3) are between the valve chambers and the "cylinder." Channel 1 carries blood to and Channel 4 carries blood away from the chambers. The action of the valves and the direction of flow is easily seen, by reference to Figs. 5 and 6, to be that of an ordinary pump with the arrangement such as to propel the blood in the direction of the arrows.

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Tubes 1 and 3 are attached by short channels (12 and 13) to the valve chambers. All three tubes are furnished with pinchcocks. The pinchcock on Tube No. 2 is close to the plates by which the tubes are compressed and is kept closed during the operation of the pump. The pinchcocks on Tubes Nos. 1 and 3 are adjustable as to height. On No. 1 it is kept low except when the tube is being compressed as an aid in filling the chamber. Tube No. 3 serves to modify the impulse given to the blood. When the pinchcock is low the blood is propelled in short violent spurts; as it is raised higher and higher the rubber tube by its cushionlike elasticity more and more softens and prolongs the spurts, until a continuous pulsating flow may be obtained without reducing the total pressure.

The intermittent current for energizing the magnet is produced by interrupting a direct current by means of a mercury switch carried by a pendulum, or by any suitable mechanism, so adjusted that the time is equally divided between compression and release.

The reservoir is shaped as shown in order to reduce to a minimum the area on which blood corpuscles might stagnate and also to keep down the volume of blood necessary to operate the apparatus. The lower narrow part of the reservoir holds about 8 c.c.; since about 4 c.c. of blood is required to fill the pump chambers and all the channels, operation can be carried on with a minimum of about 5 c.c., although it is safer to use 8 to 10 cubic centimeters. (A rat donor weighing 500 grams furnishes sufficient blood for one experiment.) Around the reservoir and the return tubes is placed a larger cylinder fitted into a tapered groove for protection. It can serve as a reservoir in case larger quantities of fluid are required.

For oxygenating the blood the upper wide part of the reservoir is employed. When a small organ is being perfused it is sufficient to fill this part of the reservoir with small glass balls and to allow the returning blood to trickle over them, while at the same time oxygen released below rises between the balls to come into contact with the blood. However, when a large volume of blood is to be oxygenated, as in the perfusion of the stomach, pancreas, and part of the duodenum, a more efficient yet simple method had to be devised. This is shown in Fig. 1. It consists of a pair of windshieldlike wipers of rubber dam which smear over the inner surface of the wide part of the reservoir the returning blood which is directed by small pieces of stainless steel against the surface. The wipers are attached to a piece of glass tubing through which oxygen is conducted and released below the wipers. The glass tubing is fitted at its upper end with a brass sleeve which turns in a bushing in the glass cover of the outer cylinder. The sleeve carries a small gear which meshes with another gear, outside the cylinder, attached by a flexible cable to the reduction gear of a small motor. The wipers make about 20 complete rotations per minute and keep the blood spread in a thin film in direct contact with oxygen.

Blood is drawn by the pump from the reservoir through Channel 1 and is discharged through Channels 4 to 6 to the arterial spout and canula and thence into the artery of the organ. Having circulated through the vessels of the organ, it is discharged as dark venous blood through the venous canula to Channels 9 and 10 and on through the venous return tube to the top of the reservoir where

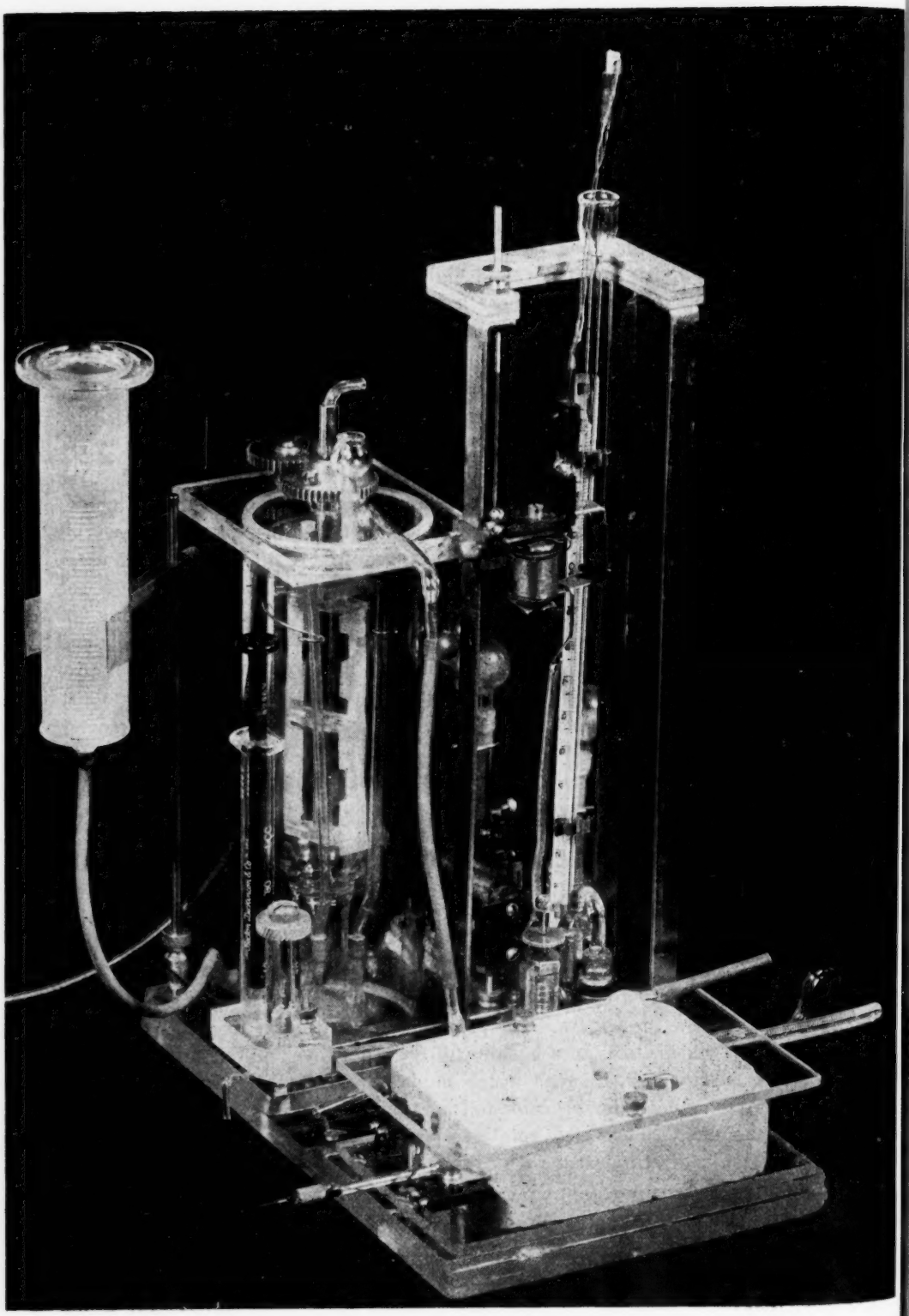


Fig. 1.

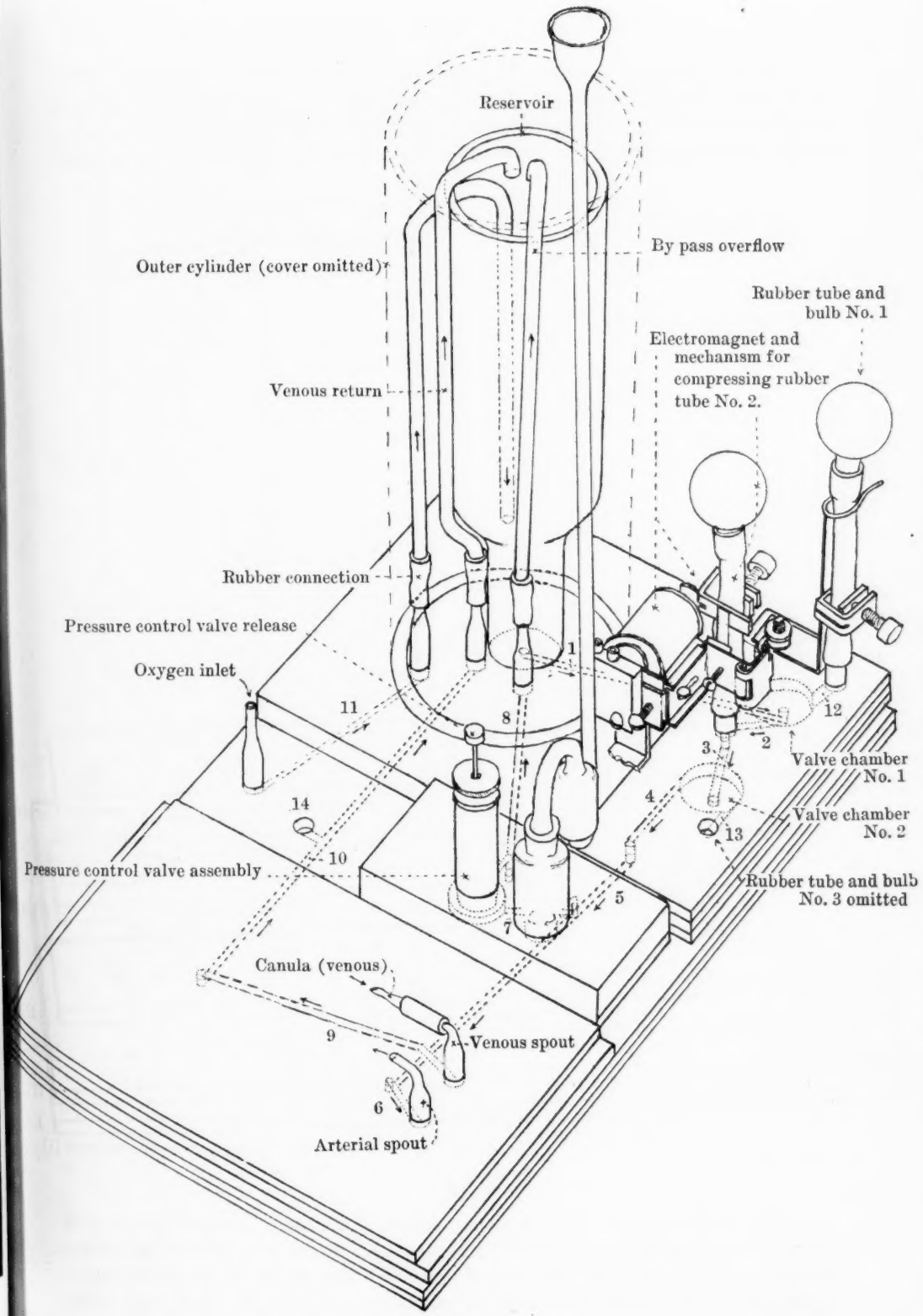
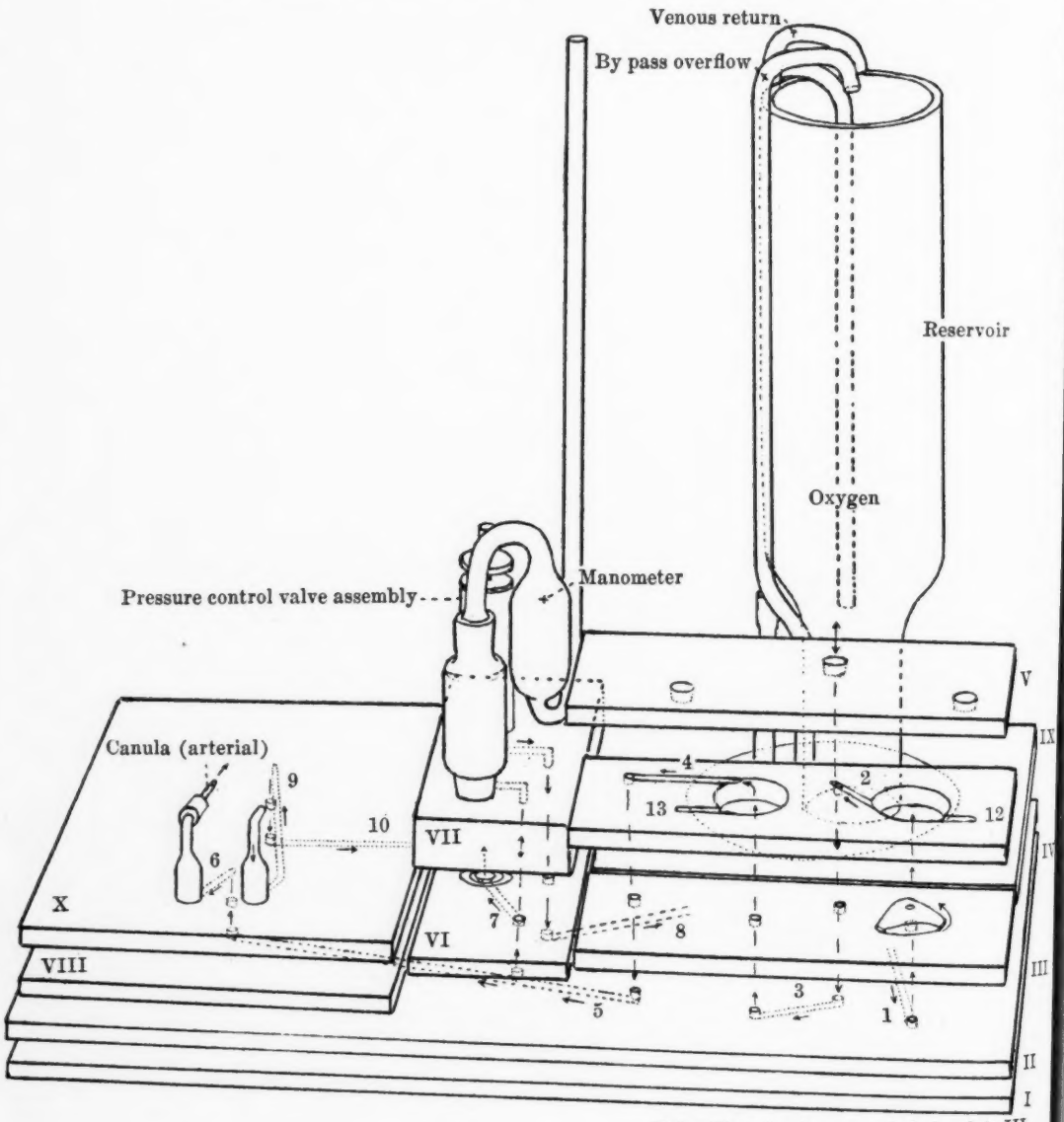

Reservoir
By pass overflow
Outer cylinder (cover omitted)
Rubber tube and bulb No. 1
Electromagnet and mechanism for compressing rubber tube No. 2.
Venous return
Rubber connection
Pressure control valve release
Oxygen inlet
1
2
3
4
5
6
7
8
9
10
11
12
13
14
Valve chamber No. 1
Valve chamber No. 2
Pressure control valve assembly
Rubber tube and bulb No. 3 omitted
Canula (venous)
Venous spout
Arterial spout

Fig. 2.

it spills over the glass balls or is spread over the inner surface. This circulation is all that would be necessary if the pump could be made to deliver just the exact amount at the desired pressure. But since the pump delivers blood in excess of the capacity of the organ, the excess must be bypassed, under control,



Valve No. 1 in place over hole in plate III

Fig. 3.

through a side channel. This is accomplished by Channel 7 which passes from Channel 5 to open under a valve (Figs. 4 and 6) which is held down by an adjustable spring in the pressure control assembly. Blood which passes this valve is carried through Channel 8 to the bypass overflow tube also to the top of the

reservoir. It is arterial blood and helps greatly to keep the blood in the reservoir oxygenated. By means of this control valve pressure in channels between the pump and organ can be adjusted to the optimum desired, for the pump is capable of giving a pressure of over 200 mm. of mercury. The pressure is indicated by a

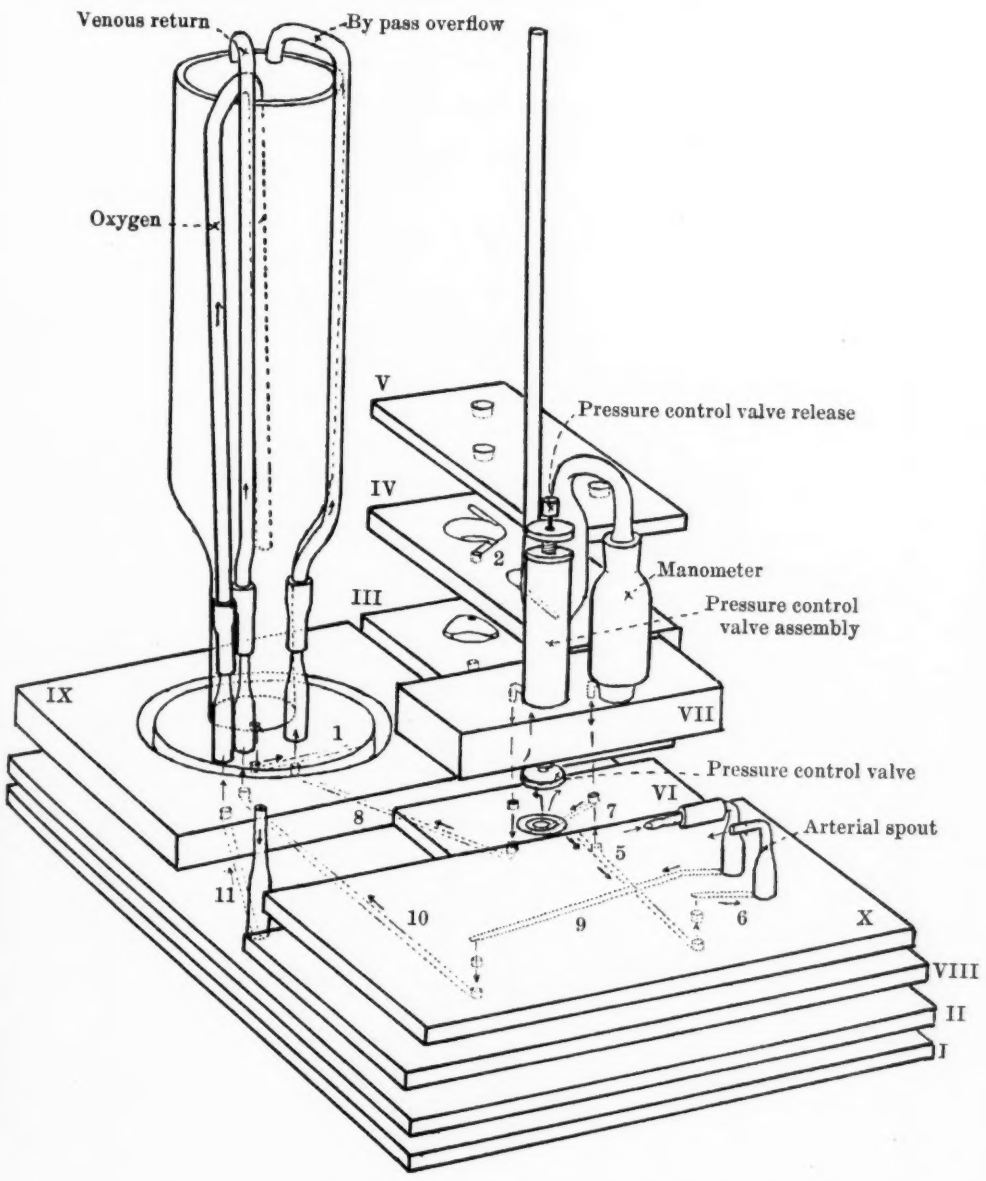


Fig. 4.

mercury manometer on another side channel between Channels 5 and 7. The arrangement of the manometer is sufficiently clear in Fig. 6, where it will be seen that there is an oil trap between the blood and mercury. It may be added that

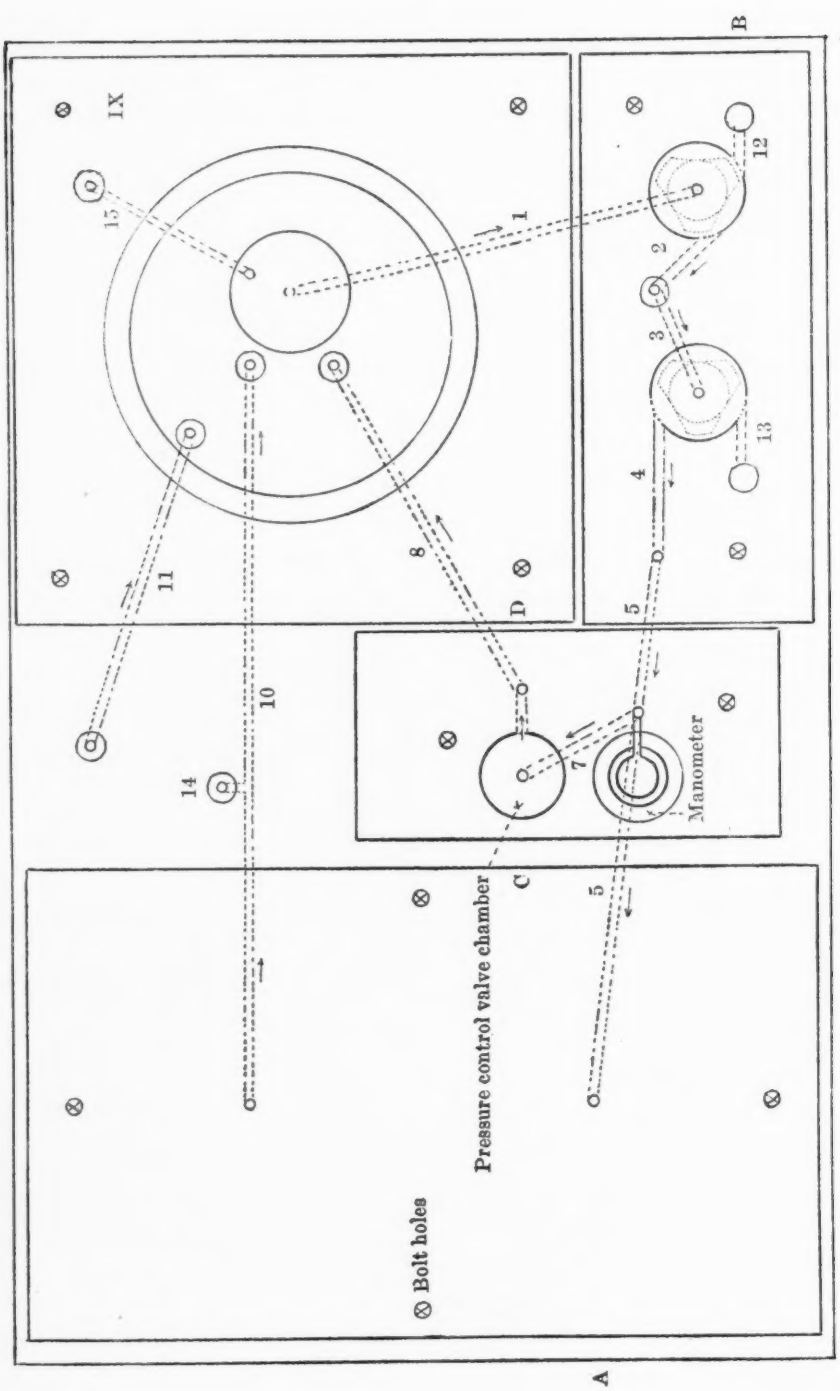

B
IX
15
1
12
2
3
13
4
11
8
D
5
10
14
7
Manometer
Pressure control valve chamber
C
5
⊗ Bolt holes
A

Fig. 5.

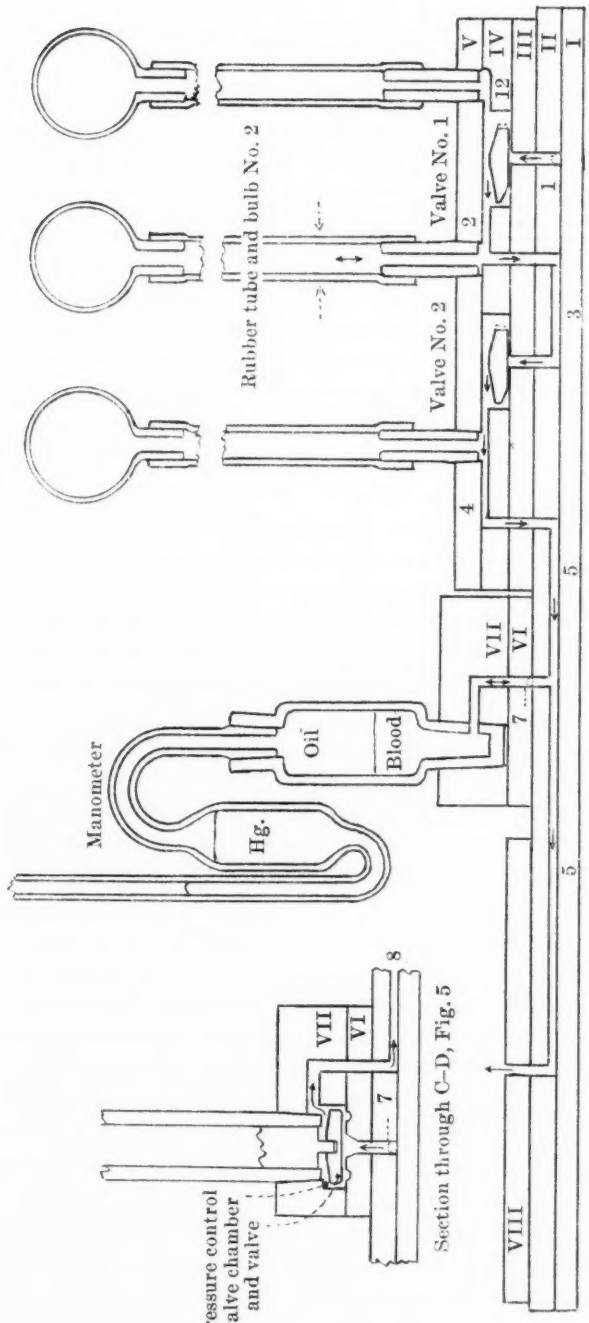


Fig. 6.—Diagrammatic section along channels and valve chambers and valves, etc. (*A* and *B*, Fig. 5).

the flask containing both blood and oil can be rotated in Plate VII, the lower portion thus serving as a stopcock when it is desired to isolate the manometer from the rest of the apparatus.

It has been found that in order to have a uniform and perfectly reliable action of the pressure control valve it is necessary to place a small disk of rubber dam between the valve and valve seat. Similar disks under the valves of the pump are advantageous.

For occasions when it is necessary to reduce the pressure quickly, a slender rod is provided attached to the valve, the raising of which at once releases the pressure in channels between the pump and specimen organ. By a simple mechanism shown in Fig. 1, this release is made automatic when the pressure becomes too high. A pair of fine enameled wires is inserted in the upper part of the manometer tube, down to the mercury level allowable. The wires are connected to an electromagnet. When the rising mercury column completes the circuit through the wires, the magnet jerks the release rod sufficiently to release the pressure.

For conveniently taking blood samples for analysis from the venous return Channel 10, at 14 (Figs. 2 and 5), is a short side channel and tapered orifice for a connecting tube or spout. The latter is connected by a short rubber tube to a three-way stockcock (Fig. 1) which, with a syringe arranged for blowing out the channels, makes it possible to take samples and to empty all channels from the outlet to Channel 10 so that each sample is uncontaminated by a preceding one.

At 15 (Fig. 5) is a channel from an outside tapered orifice, furnished with a spout, to an opening in the floor of the reservoir. To the spout a graduated cylinder is attached by a rubber tube (at the left in Fig. 1) through which the reservoir can be filled, and also emptied, with a measured quantity of blood.

Of equal importance with the rest of the apparatus are the means of supporting and attaching the organs to be perfused. This is accomplished by the specimen chamber together with Plate X on which it sets and its cover. The specimen chamber is made of plaster of Paris soaked in shellac and dried. The depressions in it are shaped (as in that for the stomach, duodenum, and pancreas, shown in Fig. 1) in such fashion as to support the organs in approximately their normal topographical positions, with provision to spread part of the pancreas over a shelf just under the cover where it can be observed under a microscope and to require the minimum amount of fluid to cover them. At appropriate locations are spouts for the blood canulae and outlets for the esophagus and duodenum. These are of stainless steel and are attached to Plate X. The outlets have connections to the outside so that fluid can be introduced into the stomach and collected from the intestine. The upper ends of the spouts appear at the right in Fig. 1. They are similar to those shown in Figs. 2 to 4 and have similar connections with the channels. In the plaster is embedded a coil of nichrome wire for heating, and two holes are provided for a thermometer and a thermostat. The size and form of the chamber and the location of the spouts differ with the organ under investigation.

In Fig. 1 may be seen a rubber and glass connection between the chamber and the upper end of the reservoir. This connection serves to return to circulation blood that accumulates in the chamber from small leaks, for it is practically impossible to prevent the escape of blood from minute tears or from seepage. The amount of this leakage varies greatly, and in no case is of importance, since it does not interfere with the supply of blood to the tissues.

The whole apparatus rests on a warm plate of approximately the correct temperature. The warm plate and the heating element and the thermostat in the chamber keep the latter at a very constant degree of heat.

The plates are sealed together with paraffin introduced when the plates are hot, with the result that all the channels, except those in the pump, are lined with a film of paraffin which keeps blood from contact with the glass, an item of some importance since most of the channels are in ordinary glass. Only certain parts are of Pyrex, namely, the pump, Plate IX into which the reservoir is set, and all parts made of tubing. In the ordinary course of sealing these Pyrex parts would not get the film of paraffin. The spouts and the reservoir are usually sealed in place with a mixture of beeswax and rosin.

The whole apparatus can be completely sterilized by appropriate methods. When it is to be thoroughly cleaned it is easily taken apart in hot water, washed with soap, and finally dried from alcohol with chamois skin before being assembled in a dustproof chamber. For work which does not require aseptic precautions it is sufficient to remove only the top plate of the pump (Plate V, Figs. 3, 4, and 6), manometer and pressure control valve and their plate (VII), the reservoir and spouts, and Plate X, along with the specimen chamber, to clean them and to rinse out the remaining channels, all of which can be done in a short time. These plates when reassembled are sealed with stopcock grease.

Since the construction of the apparatus (especially the specimen chamber, the spouts, and canulae) and the technique of isolating the organs have been developed together, it is appropriate to give a brief outline of the procedure of making the preparation. All of the dissecting must be done under a binocular microscope arranged to be focused by the feet of the operator. Minute electric cauteries, which can be turned on and off and the temperature controlled by the action of the right knee, are used almost exclusively for cutting mesenteries, vessels, and other tissues. During the isolating of the organs, which requires about one and one-fourth hours, it is important that the blood supply to organs, for example the stomach, pancreas, and that part of the doudenum to which the pancreas is attached, be maintained without interruption. The canulae used are short with bulbous tips and are attached to short pieces of rubber tubing (Figs. 2 to 4) which can be slipped over the spouts. Before being inserted into the blood vessels the free ends of the rubber connections are closed with minute stainless steel plugs, and the canulae and connections are filled with a solution of heparin. These canulae are introduced and tied into the portal vein before the latter reaches the pancreas and into the aorta just caudad to the left renal artery. Ligatures are placed in readiness around the portal vein where it enters

the liver and around the aorta cephalad to the celiac artery. Heparin is injected through the portal canula. At this stage the stomach, pancreas, and duodenum are completely separated from the rest of the body save for the aorta and portal vein through which they are receiving their normal blood supply. Also a canula has been tied into the esophagus near the stomach and the intestine prepared for attachment to its outlet. The perfusion apparatus being in readiness and the chamber partly filled with blood plasma, the ligatures on the aorta and portal vein are tied, the vessels are cut, and the organs are lifted out, rinsed in warm Locke's solution, and placed in the chamber. The plugs in the canulae connections are removed, the canulae attached the their respective spouts, the esophagus and intestine connections completed, the pump is started (usually three to seven minutes after tying off the aorta and portal), and at once the fresh blood changes the anemic appearance of the organs to that of normal health. The cover is then sealed on the chamber as shown in Fig. 1, permitting observation with a binocular microscope during the course of the experiment. This makes possible judging the condition of the organs by the appearance of the cells, blood circulation, and muscular activity.

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Scientific Program

EVALUATION OF VASOMOTOR TONE IN ANIMALS AND MAN BY MEANS OF THE TETRAETHYLAMMONIUM ION

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The blockade of autonomic ganglia produced by the tetraethylammonium ion permits evaluation of the degree of vasoconstrictor tone under various physiologic conditions.

In anesthetized dogs increasing doses of tetraethylammonium cause progressively greater depressor response until a "floor" is reached. Greater doses will not lower the pressure further. Using a dose which caused a maximum depressor response, changes in vasomotor tone were studied during alterations in blood pressure induced by (1) clamping the carotid arteries and/or vagal section ("neurogenic" hypertension), (2) continuous infusion of epinephrine or angiotonin, (3) continuous infusion of nitroglycerine, (4) hemorrhage, and (5) plethora.

During neurogenic hypertension the response to tetraethylammonium was increased, but the floor was not changed; the increased vasoconstrictor tone was completely abolished by the drug. When hypertension is induced by epinephrine or angiotonin constrictor tone is reflexly reduced in an attempt to prevent the pressure rise; the response to tetraethylammonium was diminished or absent under these circumstances.

During the infusion of nitroglycerine some degree of compensation is accomplished by reflex increase of vasomotor tone; the response to tetraethylammonium was retained and the floor was lowered. During hemorrhagic hypotension the floor obtained by an injection of tetraethylammonium was also much lower, again illustrating the presence of compensatory reflex vasoconstriction. After rapid infusion of blood the response to tetraethylammonium was diminished, suggesting a reflex inhibition of vasomotor tone during the plethoric state.

In man the drug may also be useful in assaying the role of neurogenic vasoconstriction in various vascular disorders. Tetraethylammonium causes an increase in blood flow to the extremities as measured by changes in skin temperature or by plethysmography. The increase in skin temperature is similar to that obtained after lumbar sympathectomy. When a maximum peripheral dilatation

is not obtained it suggests organic alterations in vascular supply of the extremities. It has relieved acute vasoconstrictive causalgic states.

In normotensive subjects in the supine position the depressor response to tetraethylammonium is minimal; in most hypertensive subjects the extent of the pressure fall is roughly proportional to the initial diastolic pressures. Whether or not hypertension is due to neurogenic causes it would appear that elevated constrictor tone may be a contributing factor.

Postural hypotension as evidence of loss of vasoconstrictor tone develops in both normal and hypertensive patients. It is present in some hypertensive patients who experience no drop in blood pressure in the supine position. This suggests that in some patients reflex vasoconstrictor tone is not an important factor in the elevation of the blood presssure in the supine position.

THE INFLUENCE OF VARIOUS DIURETICS ON THE URINARY SODIUM OF NORMAL PREGNANT WOMEN

W. E. Brown, M.D., and J. T. Bradbury, Sc.D. (Introduced by W. M. Fowler, M.D.), Iowa City, Iowa

Normal pregnant women were placed on a constant sodium diet, and a twenty-four hour urinary excretion of sodium (as sodium) was determined. On such a diet urinary sodium is relatively constant. A prelabor increase in urinary sodium can be demonstrated.

Studies have been made on mercury compounds, ammonium chloride, aminophyllin, glucose solutions (5 to 50 per cent), and water (6,000 c.c. daily). All of these except concentrated glucose solutions will increase urinary sodium. Concentrated glucose (25 and 50 per cent) causes a decrease in urinary sodium.

The concentration of sodium in the urine varies with the diuretic used, being greatest in the mercury compounds and lowest with water orally. These data suggest that the various diuretics differ in their effect on salt excretion and therefore have a different mechanism of diuresis. It would appear that hypertonic glucose is not indicated in toxemia of pregnancy.

DISCUSSION

Dr. Cecil Striker, Cincinnati, Ohio.—I would like to have the essayist indicate the general content of the water used.

Dr. Brown.—For intravenous solutions we used triple distilled water. The tap water in Iowa City is relatively high in sodium, and since it is the water these women drink every day there would not be much variation in our results if we used it regularly throughout this study.

Dr. John A. Layne, Great Falls, Mont.—Our group in Montana, as many of you know, has long been interested in the physiology of diuresis, and I appreciate the fact that I had an opportunity to discuss the abstract of Dr. Brown's paper with Dr. Schemm before leaving Great Falls. As far back as 1935, he and Dr. McPhail, who first published their views in 1938, held that hypertonic solutions produced a false diuresis at the expense of body water with a resultant true dehydration of the cells, which they felt only made a bad matter worse—particularly in eclampsia. This study of Dr. Brown and his associates supports this conception in so far as hypertonic dextrose is concerned.

Dr. Schemm has shown, using the less accurate method of measuring chloride excretion in the urine, that the mercurial diuretic induces a forced excretion of sodium, which is followed by a preservative suppression of sodium excretion in much the same way as the forced water excretion of hypertonic dextrose is followed by a preservative suppression of water excretion (and, we suspect, a merely coincident suppression of sodium excretion as shown in the studies reported today). It would appear futile to use hypertonic solutions, and we feel that while it is still necessary at times, it is undesirable to use mercurial diuretics whenever their use can be avoided. The other data presented today emphasize that a high volume of urinary water, with a relatively low concentration of sodium, represents the best possible mechanism for a physiologic diuresis. At the present time we know of no method more likely to achieve this ideal than a high water intake and the proper regulation of sodium ingestion.

THE MERCURIAL DIURETICS: A COMPARISON OF ACUTE CARDIAC TOXICITY IN ANIMALS AND THE EFFECT OF ASCORBIC ACID ON DETOXIFICATION IN THEIR INTRAVENOUS ADMINISTRATION

DON W. CHAPMAN, M.D., AND CARL F. SHAFFER, M.D. (INTRODUCED BY JAMES A. GREENE, M.D.), HOUSTON, TEXAS

Acute toxicity of mercurial diuretics has been noted in human beings. There are two apparently distinct immediate reactions, a nonfatal hypersensitivity and a fatal cardiac reaction. We have extended the investigation of the mechanism of immediate death in dogs from mercurial diuretics to the three commonly employed ones, Mercupurin, Salyrgan with theophylline, and Mercuhydrin.

Ascorbic acid is an extremely effective physiologic reducing agent and has been used to detoxify such chemotherapeutic heavy metals as arsenic. We have studied the possible detoxifying effect of ascorbic acid on the three previously mentioned preparations.

Mercupurin,* salyrgan with theophylline,† and mercuhydrin‡ were administered alone and in conjunction with ascorbic acid (Cenolate G§). The ascorbic acid was injected before or with these preparations at different time intervals. The seventy dogs used in these experiments were maintained in anesthesia with a 2.5 per cent solution of sodium pentobarbital and were connected with an artificial respirator. Electrocardiographic records were obtained before and during the injection. The lethal dose of a mercurial preparation was considered to be the development of a bradycardia of less than 30 beats per minute, a ventricular asystole, or a ventricular fibrillation.

All of the dogs receiving mercupurin alone or in combination with ascorbic acid died in ventricular fibrillation. All but one of the dogs given salyrgan with theophylline either alone or with vitamin C also died in ventricular fibrillation. In contrast, all but one dog when given mercuhydrin either alone or with ascorbic acid died of ventricular asystole. At present no explanation can be offered for this difference. Mercuhydrin was found to be the least toxic of the

*Campbell Products, Inc., New York, N. Y.
†Winthrop Chemical Company, Inc., New York, N. Y.
‡Lakeside Laboratories, Inc., Milwaukee, Wis.
§Abbott Laboratories, North Chicago, Ill.

three diuretics employed. Ascorbic acid when given prior to the administration of mercuhydrin or simultaneously with it increased the average minimal lethal dose over 50 per cent. The lethal dose of mercupurin and of salyrgan with theophylline was not affected when combined with ascorbic acid.

Ascorbic acid previously has been noted to increase the diuretic effect of mercupurin. A study of ten persons in congestive failure who have received mercuhydrin with ascorbic acid intravenously has revealed an equal or increased diuresis up to 50 per cent.

These investigations indicate that mercuhydrin (2 c.c.) combined with ascorbic acid (500 mg.) is the least cardiotoxic and most potent intravenous diuretic preparation studied.

DISCUSSION

DR. EDWARD MASSIE, St. Louis, Mo.—I would like to ask whether vitamin C may be given without danger in the same syringe?

DR. JOHN W. SCOTT, Lexington, Ky.—I have found clinically that the action of the mercurial diuretics can be smoothed out by giving the patient one of the B complex lyophilized preparations dissolved in the mercurial product. This makes a clear solution which has been perfectly harmless in the 100 or more instances in which I have used it.

DR. CHAPMAN.—The workers in South America have used magnesium sulfate. While their report on results so far as percentages are concerned has not been given, there was some decrease in toxicity when these products were used in combination.

We have given vitamin C and the mercurial product in the same syringe with no bad effects.

Our experience has been the same as Dr. Scott's in the use of vitamin B complex together with the mercurial substance; it does help to smooth the use of the mercurial diuretic, clinically at least.

THE SERUM PROTEIN FRACTION RESPONSIBLE FOR THE THYMOL TURBIDITY TEST

PHILIP C. COHEN, M.D., AND FRANCES THOMPSON, B.S. (BY INVITATION), MADISON, WIS.

The thymol turbidity test of Maclagan (1944) has been shown to be of considerable diagnostic value in cases of liver disease. Maclagan stated in his paper that the test was probably due to an increase in serum gamma globulin. Recant and associates (1945), on the other hand, have shown that the test depended on the presence of a lipid-containing fraction of serum globulins rather than on the gamma globulins.

A systematic study in this laboratory of the serum protein fraction responsible for the thymol turbidity test indicates that a beta globulin is involved. A large number of fractions obtained from pooled normal human plasma were tested with the thymol reagent. High values were always associated with those fractions containing a high percentage of beta-1 globulin. Electrophoretic analysis of sera showing high thymol turbidity values invariably showed a high beta globulin level, whereas sera having normal beta globulin levels gave normal turbidity values. After mixing positive sera with thymol reagent and then dialyzing against the thymol reagent, it was possible to centrifuge off the precipitate from the supernatant and examine both in the electrophoresis ap-

paratus. The serum after treatment with thymol reagent, and free of its precipitate, differed from the untreated serum chiefly in a decrease of the beta globulin fraction to a normal level (Table I). Further, electrophoretic analysis of the precipitate after redissolving showed it to be chiefly a single protein with an electrophoretic mobility characteristic of normal beta globulins.

TABLE I. ELECTROPHORETIC ANALYSIS OF SERUM COMPONENTS BEFORE AND AFTER TREATMENT WITH THYMOL REAGENT

	PER CENT COMPOSITION				
	ALBUMIN	ALPHA-1	ALPHA-2	BETA	GAMMA
Before	47.7	4.5	9.1	19.1	19.5
After	53.3	5.5	9.1	13.0	19.0

While the beta globulin fraction of blood is known to be lipid-containing, the total lipid content of sera did not appear to be related to the thymol turbidity values. Thus, a serum from a case of lipoid nephrosis with a cholesterol content of 1,400 mg. per 100 c.c. showed a negative test.

In a comparative study of cases of liver disease showing a discrepancy in the thymol turbidity and cephalin-cholesterol flocculation tests, the latter was invariably positive in cases of high gamma globulin levels and the former in cases of high beta globulin levels. In cases where both tests were positive both these fractions were elevated above normal levels. In general it was found that higher and more consistently positive thymol turbidity values were associated with acute inflammatory rather than chronic degenerative hepatic disorders.

DISCUSSION

DR. WILLIAM S. HOFFMAN, Chicago, Ill.—It would appear from Doctor Cohen's data that a positive thymol turbidity test reduces the beta globulin concentration of the serum to a level below normal. I wonder if this indicates that the positive thymol turbidity test is produced by the presence of a substance which, when thymol is added, precipitates the normal beta globulin.

BIOPSY OF LIVER IN PATIENTS WITH ACTIVE BRUCELLOSIS: DESCRIPTION OF HEPATIC LESIONS

FREDERICK W. HOFFBAUER, M.D., AND WESLEY W. SPINK, M.D.,
MINNEAPOLIS, MINN.

A review of the literature on the alterations of the tissues in patients with brucellosis indicates that the changes are widespread involving, particularly, the reticulo-endothelial system. Practically all the reports have been based upon an examination of post-mortem material. Only on rare instances have biopsies of the liver been made in living subjects. The present report is part of a study on the pathogenesis of brucellosis and represents an endeavor to define the incidence and nature of the hepatic lesions in patients with active brucellosis.

Thus far, one patient with acute brucellosis and four with the chronic form of the disease have been studied. Four of the patients were proved to have brucellosis by isolation of *Brucella abortus* from the blood stream. The fifth individual had clinical and laboratory findings consistent with chronic brucellosis. In one subject a specimen of liver was obtained by means of peritoneoscopy;

in a second individual material was acquired incidental to an abdominal operation; and in the three remaining patients biopsies were made by an operative procedure under local anesthesia.

Abnormal histologic findings were apparent in all specimens. The liver of each of the five patients showed the presence of granulomatous lesions which have been described in brucellosis. The granuloma were usually found in the liver cords rather than in the portal spaces. These lesions were composed of epithelioid cells, lymphocytes, and plasma cells arranged in small round groups. In addition, variable increases in portal connective tissue and occasional necrotic liver cells were seen.

In an endeavor to correlate the hepatic injury with liver function, multiple liver function tests were carried out which included quantitative serum bilirubin, bromsulfthalein excretion, cephalin cholesterol, thymol turbidity, urine urobilinogen, and urine coproporphyrin. The degree of functional impairment in the five cases studied was slight contrasted with the extensive histologic changes. These observations indicate that hepatic changes may occur more frequently than is generally recognized in patients with active brucellosis.

STUDIES IN CONVALESCENCE FOLLOWING HERNIORRHAPHY: THE EFFECT OF DIET, SUPPLEMENTS, AND AMBULATION ON METABOLIC CHANGES AND ON THE PERFORMANCE OF PATIENTS AS MEASURED BY TESTS OF CARDIOVASCULAR EFFICIENCY AND MUSCULAR FITNESS

R. W. Keeton, M.D., W. H. Cole, M.D., N. O. Calloway, M.D., N. Glickman, M.S., H. H. Mitchell, Ph.D., J. Dyniewicz, Ph.C., and D. Howes, B.S., Chicago and Urbana, Ill.

An attempt was made to determine the extent and duration of convalescence from surgical trauma and to find means of shortening it. The progress of convalescence was measured by a study of certain metabolic factors and performance tests.

The liver was the organ primarily affected in ninety-seven healthy men submitted to herniorrhaphy. The decrement in liver function, as measured by twenty-four hour urobilinogen excretion, bromsulfalein retention, and other tests, lasted about five days and returned slowly to normal. The decrement was considerably greater in patients who developed complications with infections in chest or wound. The decrement could be prevented by high protein and high caloric feedings, combined with ambulation, systematic exercises, and the administration of certain supplements.

The negative nitrogen balance usually observed postoperatively in patients of the control group on the usual hospital regime was prevented by the same measures which prevented the decrement in liver function. Ambulation alone caused a shift in nitrogen balance toward the positive side.

Attempts to use 17-ketosteroid excretion, creatine excretion, creatinine excretion, renal tests, and many additional liver-function tests were of little clinical value.

The cardiovascular efficiency was measured by the response of the patient on the tilt-table, stair-climbing test, step-up test, and an over-the-bed, push-up test. A single, numerical value has been developed for expressing the cardiovascular response to tilting.

Patients who were on the program of full ambulation exhibited less cardiovascular impairment than those on a program of modified ambulation or those on the control program. Patients who received adequate quantities of proteins and calories throughout the postoperative period (tube fed) also showed less impairment than the controls. This indicates that adequate proteins and calories exerted a favorable effect on circulatory efficiency.

The strength of hand grip, the endurance time of hand grip, and the response to selected psychomotor tests showed no significant decrements.

The expected temperature and pulse rate variations of patients exhibiting normal convalescence will be illustrated and described.

The totality of the results indicates that a herniorrhaphy is a slight operative load. It is a more useful procedure for studying metabolic changes than those revealed by performance tests. It is an excellent experimental procedure, since it is possible to secure adequate controls and the patients can all be brought to a normal state of health prior to operation.

DISCUSSION

DR. RICHARD M. JOHNSON, Long Beach, Calif.—I want to ask what you mean by high caloric intake. There seems to be some doubt if nitrogen balance can be maintained on less than 1,500 calories a day, so I would like to find out how many calories you give. I think that this is an important study, and in any study of this sort it is important to know how much activity the patient has and how much his muscles are used.

DR. M. A. BLANKENHORN, Cincinnati, Ohio.—I would like to ask the essayist if he has compared all wound infections with infections of the lung with atelectasis. I have in mind that a lung infection might result in an anoxia. This might be an important determining factor in the urobilin output. I would also like to ask if these were all urine urobilin determinations and whether any accounting was made of the stool urobilin.

DR. WARREN B. COOKSEY, Detroit, Mich.—Are there any preliminary reports on more prolonged or more chronic illnesses? I did not understand whether these patients were in the younger age group or whether they were an army group. Certainly Dr. Keeton and his coworkers deserve our commendation. I have the feeling that clinically an organized convalescence is of great value, but I know how difficult it is to get any support for a program of this sort. I can testify to this after seventeen years of attempting through subsidy and endowment to create a really good convalescent facility for Detroit, which is, I believe, a typical American city in this respect. We are barely able to keep a thirty-five bed endowed institution full of true convalescents. We are flooded with chronic and senile cases. We must pay more attention to the convalescent problem, and Dr. Keeton's work is an excellent start.

DR. CECIL J. WATSON, Minneapolis, Minn.—I judge that there is less urobilin excreted in patients who have been given spinal anesthesia. What type of inhalation anesthesia was used in the other patients?

DR. KEETON.—I am quite certain that the problem of calories is important. We regard the basal caloric requirement plus 20 per cent as a maintenance or just maintenance diet. As a matter of fact, these patients lost weight on that diet. When we talk about high calorie diets we mean basal plus 100 per cent. If the basal requirements are 1,700 calories and the

patient is fed 3,400, that should be more than sufficient to take care of the increased requirements associated with operation.

As to the age group of these patients, they were very largely young men who were furnished to us through the draft boards. Consequently, we believe that this constitutes a good control group for the study of operations because the subjects were relatively normal. I appreciate the suggestion made that when we start on most operations we are working on sick individuals and it becomes more difficult to study the patient's physiology prior to his operation.

We have not made any careful study of patients' response based on the types of infection. I simply gave you all the infections which arose in the course of this operation. We have recently agreed that in order to standardize further our procedures we would use penicillin as a prophylaxis for postoperative pulmonary infections.

The anesthetic which was used was nitrous oxide induction with ether. Pontocaine was used for the spinal anesthetic. No determinations were made of the urobilinogen in the stools.

RELATION BETWEEN STRUCTURAL AND FUNCTIONAL ALTERATIONS OF THE LIVER

HANS POPPER, M.D. (BY INVITATION), MURRAY FRANKLIN, M.D. (BY INVITATION), FREDERICK STEIGMANN, M.D., AND DONALD D. KOZOLL, M.D., CHICAGO, ILL.

In eighty-five patients suffering from various hepatic diseases (infectious and toxic hepatitis, cirrhosis, benign and malignant extrahepatic obstruction, and other hepatomegalies), liver biopsies were performed by needle; they were repeated in twenty-seven instances, sometimes by surgical excision. Prior to each biopsy several liver function tests were performed. In general, the histologic diagnosis agreed with that derived from clinical and laboratory observations. Occasionally, the histologic differentiation of biliary hepatitis due to extrahepatic obstruction from infectious and particularly toxic hepatitis was difficult. Sometimes the clinical impression was corrected by the histologic findings.

To evaluate morphologically the liver function tests, the degree of each histologically demonstrable change was plotted against the result of each test and statistically evaluated. From the material examined so far, a close correlation appeared between diffuse damage of the epithelial liver cells (characterized by histologic criteria) and pathologic results in cephalin cholesterol flocculation, thymol turbidity, and bromsulfalein tests; between moderate relation to reduction of prothrombin time, increase of serum bilirubin, and reversal of A/G ratio; and between slight relation to increase in sedimentation rate, urinary urobilinogen, N. P. N., and decrease in cholesterol ester ratio and serum vitamin A. No quantitative relation appeared to serum alkaline phosphatase, total cholesterol, fecal urobilinogen, and direct-indirect bilirubin ratio.

In contrast, focal parenchymal necrosis bore a slight relation only to cephalin flocculation, thymol turbidity, and serum vitamin A, indicating that focal damage, even if more severe than diffuse liver cell damage, may escape recognition by function tests. Regeneration of the liver cells showed a significant relationship to thymol turbidity and a questionable one to cephalin flocculation and serum vitamin A. Regeneration was more often found when drop

in cholesterol esters and A/G reversal were moderate than if advanced. Reconstruction of the lobular pattern appeared related to cephalin flocculation, less to thymol turbidity. With marked inflammatory activity in the periportal fields, the sedimentation rate was uniformly elevated; the former bore some relation to cephalin flocculation, increased serum bilirubin, and reduced prothrombin time. Its relation to thymol turbidity and urinary urobilinogen was equivocal. Increased activity of Kupffer cells coincided only somewhat with A/G reversal, reduced cholesterol esters, increased serum bilirubin, and elevated thymol turbidity.

Although obviously marked overlapping occurs between histologic changes in the individual patient, it is nevertheless hoped that this morphologic approach may help in the diagnosis of hepatic disease by establishing relations between results of function tests and the different pathologic phenomena within the liver.

DISCUSSION

DR. CECIL J. WATSON, Minneapolis, Minn.—Dr. Hoffbauer and I often have been impressed with the striking lack of correlation between histologic evidence of disease and changes as reflected by some of the functional tests. This was first borne in upon us several years ago in studying liver damage due to sulfanilamide. In many of these cases we saw outspoken jaundice with other evidence of liver dysfunction but with surprisingly little histologic evidence of impairment. Conversely, we often have seen instances of cirrhosis of the liver in which there was but little evidence of functional impairment in spite of marked histologic derangement.

I should like to emphasize that group of cases of late or chronic hepatitis with jaundice which is perhaps best designated as cholangiolitic hepatitis, in which liver cell function has returned to normal and the appearance of the liver cells as studied by means of biopsy is found to be normal. One often sees little in the biopsies from this sort of case, although the condition is inflammatory. Because of marked evidence of regurgitation of bile in the blood, one can only conclude that there is a functional injury of the cholangiolar epithelium.

I notice that Dr. Popper has used a simple Ehrlich test and I presume that the test was carried out on single samples or short period collections of urine. I think the upper limit of normal which he gives as 3 units is too high. We studied sixty normal students and in that group found that 95 per cent had less than 1 unit in two-hour samples from 2 to 4 in the afternoon; the rest ranged up to 1.5 units. We are inclined to believe that 1.5 units represent the upper limit of normal, although there may be borderline cases up to 2.0 units. We have regarded anything over 2 units as distinctly abnormal.

I should like to emphasize that this procedure has limited value. It has a good deal of value because of its simplicity and the ease with which it can be applied to a large number of individuals. On the other hand, we have seen individuals with liver disease who had a normal value over a short period of time, and yet in the same twenty-four hour period the urobilinogen might show a considerable degree of elevation.

DR. FREDERICK W. HOFFBAUER, Minneapolis, Minn.—I should like to compliment Dr. Popper and his associates on this study. I would like also to ask about the distinction between extrahepatic biliary obstruction and hepatitis on the basis of biopsy. We have thought that if the biopsy could be secured early in the course of the jaundice the distinction would be possible, but when the biopsy is made ofter the patient has been jaundiced for three or four weeks, the pathologist will have considerable difficulty distinguishing between the changes that occur in extrahepatic obstruction and those that occur in hepatitis.

I should like also to ask about the degree of depression of cholesterol esters in patients with biopsy evidence of fatty livers. I am sure the surgeons would welcome some evidence

that would denote or at least lead them to suspect fatty infiltration of the liver. How extensive were the changes in the cholesterol ester fractions?

DR. LEON SCHIFF, Cincinnati, Ohio.—I would like to ask Dr. Popper if he has noticed any specific changes in the livers of those patients with hepatitis in whom the jaundice appears to be completely obstructive in character. These cases present laboratory changes that cause them to be confused with cases of obstructive jaundice due to stone or tumor. Is there any specific change in the liver biopsy specimens in this particular group of hepatitis often called cholangiolitic form of hepatitis?

DR. POPPER.—First, I want to stress once more that in a certain number of patients examined, discrepancies between anatomical and functional changes were found. For instance, a certain percentage of patients who revealed liver function impairment did not show liver cell damage in the biopsy specimen and vice versa. What we are attempting is a statistical approach comprising all cases.

We can confirm the observations of Dr. Watson as to the occurrence of the morphologic picture of cholangiolitic cirrhosis without significant disturbance of many of the liver functions. We have five cases of this type on record.

As to the question of histologic differentiation between the various types of hepatitis, we found the morphologic differentiation between infectious (viral) hepatitis from toxic hepatitis due to a variety of causes not too difficult to make. We believe we have histologic criteria as well for the fatal as for the nonfatal form of these diseases. On the other hand, the differentiation between toxic hepatitis from biliary hepatitis due to extrahepatic obstruction is sometimes difficult. We have made most of our mistakes in the interpretation of liver biopsies along this line. We are trying, at present, to develop histologic criteria by obtaining additional experience on those cases. One reliable evidence for a biliary hepatitis due to extrahepatic obstruction is the so-called bile infarct which represents necrotic areas impregnated with bile. It does not occur in other groups. We are at a loss to explain the morphologic cause of intrahepatic biliary obstruction and we accept the explanation of Watson and Hoffbauer, namely, that of a morphologically unrecognizable reflux through the bile ducts.

We are encountering difficulties in the chemical determination of cholesterol esters in using the colorimetric method. In our material fatty changes were often accompanied by a decrease in the cholesterol ester percentage. On the other hand, we occasionally saw severe hepatitis with little decrease of the ester percentage.

HEPATITIS WITHOUT JAUNDICE IN INFECTIOUS MONONUCLEOSIS

QUIN B. DEMARSH, M.D. (BY INVITATION), AND HOWARD L. ALT, M.D.,
CHICAGO, ILL.

From reports of punch biopsies, the jaundice occasionally associated with infectious mononucleosis has been shown to be due to parenchymatous changes in the liver not unlike those seen in infectious hepatitis. Similar changes in the liver have been reported in a nonjaundiced case of infectious mononucleosis. Cohn and Lidman (1946) found evidence of impaired liver function in fifteen consecutive cases of infectious mononucleosis without jaundice.

We have performed serial liver function tests in nineteen consecutive cases of infectious mononucleosis without jaundice (icterus indices of 10 units or less). Some degree of hepatic dysfunction was present in all cases as shown by the cephalin-cholesterol flocculation and sulfobromphthalein excretion tests. The maximum dye retention was 36 per cent in one hour. An abnormal albumin-globulin ratio was present in two out of ten cases in which it was determined.

There was a correlation between the severity of symptoms and the degree of liver damage. The usual duration of hepatic dysfunction was three to six weeks, but in three cases it persisted for three to four and one-half months. The liver was palpable and/or tender in eight of the nineteen cases.

The treatment consisted of rest, a high protein, high carbohydrate diet, and vitamin supplements. We have no evidence that the course of the disease was influenced by the diet. Patients who resumed moderate activity before the liver function returned to normal had no relapse of liver disease as indicated by liver function tests or by an increase in size and tenderness of the liver.

DISCUSSION

DR. A. E. FELLER, Cleveland, Ohio.—How was the diagnosis established in these cases?

DR. DEMARSH.—We performed serial white blood cell and differential counts in all of these patients. We made the diagnosis clinically first and confirmed it by heterophil agglutination tests. We did not do the absorption test. The clinical picture in each case was very typical and could not be confused with anything else.

THE EXPERIMENTAL PRODUCTION OF JAUNDICE OF THE DIRECT-REACTING TYPE BY THE INJECTION OF A PREPARATION OF DIRECT-REACTING BILIRUBIN

F. E. SNAPP, M.D. (BY INVITATION), M. GUTMANN, M.S. (BY INVITATION), T. W. LI, PH.D. (BY INVITATION), AND A. C. IVY, PH.D., CHICAGO, ILL.

By making an alkaline aqueous solution of crystalline bilirubin and mixing it with pooled canine plasma, and then injecting this alkaline bilirubin-plasma mixture into dogs, a direct-reacting type of jaundice, as occurs in obstructive jaundice, can be produced.

The amount of plasma and source of bilirubin is important; the best mixture is made as follows: 300 mg. of crystalline gallstone bilirubin is dissolved in 45 c.c. of N/10 NaOH, and the resulting solution is added to 300 c.c. of pooled reconstituted canine plasma. When this is given slowly intravenously, no reactions or changes in body temperature have been observed in twelve dogs. For some unknown reason about twice as much bile bilirubin as gallstone bilirubin has to be used to give an equivalent blood retention of the bilirubin (thirty-four tests).

By giving an injection of 200 to 300 mg. of bilirubin plasma every third or fourth day, or smaller doses every day, the jaundice was maintained in three dogs for four weeks. The jaundice cleared in five to seven days after ceasing the administration.

When the jaundice was maintained by giving 50 mg. of bilirubin in 50 c.c. of plasma daily, the only change detected was a delay in rose bengal clearance. With larger doses, the rose bengal clearance was decidedly reduced in three dogs, the alkaline phosphatase was slightly increased in one, the sedimentation rate of erythrocytes was markedly increased in two and slightly in the other, and in four weeks a slight anemia occurred. The animals ate well and did not lose weight.

It is suggested that a method is now available for producing a direct-reacting type of jaundice in patients with arthritis by substituting human for canine plasma in the previously mentioned specifications with the idea of ascertaining whether a remission of the disease may be induced.

DISCUSSION

DR. CECIL J. WATSON, Minneapolis, Minn.—If this method were to be applied in a large series of cases it would be interesting to compare the two forms of jaundice which one might expect, that is, that which is produced immediately due to the injection of bilirubin and that which will ensue in some instances about three months later due to transmission of the virus of homologous serum hepatitis.

THE EFFECT OF METHIONINE UPON THE URINARY NITROGEN IN HUMAN BEINGS AT NORMAL AND LOW LEVELS OF PROTEIN INTAKE*

RICHARD M. JOHNSON, M.D., H. J. DEUEL, JR., PH.D. (BY INVITATION), MARGARET G. MOREHOUSE, PH.D. (BY INVITATION), AND JOHN W. MEHL, PH.D. (BY INVITATION), LONG BEACH, CALIF.

Methionine offers considerable protection to the livers of experimental animals against hepatoxic agents and is thought to be of some therapeutic value in the treatment of certain types of liver disease in man. Methionine has been observed to exert a protein-sparing action in the dog, rat, and chick. This study was undertaken to determine the protein-sparing action of methionine in man.

Eight volunteers were placed upon diets containing 2,000 calories and 11.9 and 2.3 Gm. of nitrogen with and without 3 Gm. d-l methionine for one or more periods of ten days each.

All subjects were constantly observed by four nurses and an attendant who kept daily records of temperature, pulse, respiration, and physical activity and weekly records of body weight, blood pressure, plasma protein concentration, hemoglobin, and red blood cell counts. The daily urinary excretion of nitrogen, creatinine, and sulfate was determined and stools were weighed. The diets were analyzed for their nitrogen content.

Methionine did not significantly alter the urinary nitrogen or creatinine excretion in any subject under these conditions. About 80 per cent of the methionine given were accounted for by increased urinary sulfate excretion and 10 per cent were excreted as methionine in the urine.

Four volunteers from the Department of Biochemistry were given 600 calories or 1,200 calories containing 0.94 Gm. nitrogen. In this group methionine failed to exert a protein-sparing action. Ninety-six per cent of the methionine taken was accounted for by increased sulfate excretion.

Conclusions.—Methionine did not exert a protein-sparing action at 2,000 caloric intake level containing 11.9 and 2.3 Gm. of nitrogen.

Methionine did not exert a protein-sparing action at a 600 or 1,200 caloric level containing 0.94 Gm. nitrogen.

The methionine was metabolized.

*This work was done under a contract between the University of Southern California and the Research and Development Branch, Military Planning Division of the Office of the Quartermaster General, United States Army.

A species difference for minimum requirements of methionine is suggested. The daily urinary nitrogen excretion was lowest at the 2,000 caloric intake level and greatest at the 600 caloric intake level.

ULCER OF THE LEG DUE TO ARTERIOLOSCLEROSIS AND ISCHEMIA OCCURRING IN THE PRESENCE OF HYPERTENSIVE DISEASE (HYPERTENSIVE-ISCHEMIC ULCERS)

A Preliminary Report

Edgar A. Hines, Jr., M.D., and Eugene M. Farber, M.D. (By Invitation), Rochester, Minn.

A small group of patients has been observed who had painful ulcers of the legs which were difficult to heal and which did not fit into any of the usual clinical classifications of ulcers of the leg. The lesions did not have the appearance of typical stasis ulcers but were ischemic and similar to the ulcers which result from spastic or occlusive involvement of the smaller arteries such as occurs in chronic pernio, livedo reticularis, or senile changes in the skin. However, in this particular group of patients none had any of the commonly recognized conditions producing ischemic or stasis ulcers, and no dermatitis was present. The usual diagnosis was ischemic ulcer of undetermined cause.

After observation of several patients it was noted that, aside from the fact that they were all women with the same type of ulcer of the legs, they had one condition in common, namely, hypertensive disease of long duration and considerable sclerosis of the retinal arterioles of the chronic hypertensive type. It was considered that changes similar to those in the retinal arterioles could also occur in the small arteries of the skin and subcutaneous tissues and could give rise to small areas of infarction of the skin. As the result of trauma or for some unknown reason, the skin might break down and such an ischemic ulcer would form.

The first case in which we considered that the ulcer on the leg might be related to vascular changes in the skin due to long-standing hypertensive disease was observed in 1941. Up to the present time we have observed seven patients and have obtained from the file of the clinic records of four patients who had the typical ulcers and hypertensive disease of long duration and no other vascular or cutaneous disease which could account satisfactorily for the lesions. We are presenting this group of eleven cases as a possible new syndrome, with the hope that additional observation and study will further clarify the cause and treatment of these lesions.

Histopathologic study of specimens from the ulcers and of the adjacent skin in five cases revealed organic changes in the arterioles. The most common changes were an increase in the thickness of the arteriolar wall and a decrease in the diameter of the lumen. An increase in the number and size of the nuclei and hyaline degeneration in the media, intimal proliferation, and periarteritis were also noted. The changes paralleled those reported by Keith, Wagener, and Barker from their studies of the pectoralis major muscles in cases of essential hypertension. There were no significant pathologic changes in the sections from the tissue removed from the ulcers except with reference to the arterioles.

Tentative criteria for the diagnosis of hypertensive-ischemic ulcer of the leg should include: (1) hypertension, (2) an ischemic appearing ulcer, (3) indolence of ulcer, (4) moderate to severe pain, (5) poor response to conventional treatment, and (6) typical changes of the blood vessels in and near the lesion as indicated by biopsy. The following should be ruled out before the diagnosis is made: (1) significant chronic venous insufficiency, (2) occlusive arterial disease, (3) syphilis, (4) blood dyscrasia, (5) cutaneous sensitivity to drugs, frostbite, seasonal variations affecting the occurrence of the lesions, or serious local injury, and (6) other diseases on pathologic study of the lesion.

DISCUSSION

DR. ROY W. SCOTT, Cleveland, Ohio.—Will you tell us what therapeutic management you have found of most value in these cases?

DR. A. A. HOLBROOK, MILWAUKEE, WIS.—Several of the photographs of leg lesions looked like cutaneous diphtheria. I wonder if any culture studies were made.

DR. HINES.—In regard to treatment, we have considered these patients from the standpoint of an occlusive arterial disease. Treatment has included such vasodilators as heat or diathermy applied to other parts of the body than the lower extremities, typhoid vaccine administered intravenously to produce fever in the younger patients, and occasionally whisky given orally. I believe this approach has been helpful. Of the various local applications tried, and they have been numerous, it would seem that powdered blood cells worked the best. The ulcers were slow to heal but they did heal eventually.

Cutaneous diphtheria was considered in several cases. Cultures were made several times in each case and we found no special organisms in these cultures. I do not believe the condition can be considered as cutaneous diphtheria.

AORTIC STENOSIS: AN ANALYSIS OF 106 PROVED CASES

CARL KUMPE, M.D. (BY INVITATION), AND WILLIAM B. BEAN, M.D.
CINCINNATI, OHIO

Recently two instances of advanced aortic stenosis with bizarre clinical findings were studied during a single week on the wards of the Cincinnati General Hospital. Because the diagnosis was missed we have undertaken a review of all cases of aortic stenosis without significant lesions of other valves which have come to autopsy in the Department of Pathology in the last twenty-six years. We found 106 cases from a total of 15,806 records. A survey of medical reports and textbook discussions of aortic stenosis revealed great emphasis upon the classical signs of basal systolic murmur transmitted upward, and thrill, but little or no consideration of the atypical case which in our experience has actually predominated. Correct diagnosis, unless established in a former admission, was rarely made in the presence of acute cardiac failure of either forward or backward variety because of the absence of classical signs. The most common mistakes in diagnosis were primary disease of the central nervous system (that is, cerebral vascular accident, epilepsy), acute myocardial infarction, and pulmonary embolism. Even when the diagnosis of aortic stenosis was correct, mitral stenosis was diagnosed frequently but not found post mortem.

The history, mode of onset, electrocardiogram, x-ray, clinical and laboratory data, course of the disease, and manner of death will be correlated with the

cardiac and other findings at autopsy. Although syncopal attacks and even transient hemiparesis were fairly frequent, dizziness and petit mal-like seizures were very common as were anginal pain and paroxysms of dyspnea. A terminal state of shock with symptoms referable to a variety of organs was encountered often. Advanced degrees of aortic stenosis were found to be compatible with a relatively long life, especially if the patient lived within the narrow margins of his cardiac reserve. With these points in mind recent experience has shown that the diagnosis of aortic stenosis can be made without the classical signs if the possibility is kept in mind. In fact the local trend now is in danger of swinging too far.

DISCUSSION

Dr. Roy W. Scott, Cleveland, Ohio.—The difficulties in making a definite diagnosis in patients with congestive heart failure are well established. We all have missed these patients and have been surprised to find very marked aortic stenosis at the post-mortem table. I hope that Dr. Kumpe will comment on the question of whether calcific aortic stenosis is a distinct entity or whether it is always due, as some believe, to a preceding inflammatory process involving the aortic leaflets.

Dr. Kumpe.—In reference to the question about calcific aortic stenosis we do not have any answer. We did not go into the pathology. Dr. Bean agreed that it was a good topic to avoid.

In reference to the question about pure congenital aortic stenosis, no case was considered to be congenital in this series.

As to the electrocardiographic changes, forty-four showed left axis deviation, seven no axis deviation, and one a transient early right axis deviation which later returned to no axis; there were twelve auricular fibrillations, and there was a P-R interval in twenty-one cases over 0.2 second.

AN ANALYSIS OF IMMEDIATE MORTALITY IN 572 CASES OF RECENT MYOCARDIAL INFARCTION

L. N. Katz, M.D., and S. S. Mintz, M.D. (By Invitation), Chicago, Ill.

A series of 572 cases seen at the Michael Reese Hospital between 1940 and 1945 inclusive have been investigated to determine some of the factors involved in causing immediate mortality (during the hospital stay). Hypertension appears to have no influence on the immediate prognosis of acute myocardial infarction. Diabetes mellitus definitely adds to the gravity of the prognosis, especially in women. It increases the frequency of death by precipitating uncontrolled diabetes and ketosis. Angina pectoris preceding acute infarction has no significant effect on the immediate prognosis. However, the persistence of anginal pain after the attack makes the immediate prognosis graver. Pulse pressures of 25 mm. Hg or less are of great prognostic significance. A systolic blood pressure drop to 90 mm. Hg for several days is a poor prognostic sign. The presence of shock has a similar implication. In the hypertensive group a drop of systolic pressure to 100 mm. Hg for three to five days makes the immediate prognosis poor. The immediate mortality is greater in patients having an infarct involving the septum or one showing atypical or combined patterns. There is no significant difference in the mortality rate of anterior and posterior wall infarcts. In all types the mortality rate is higher in women than in men. Cardiac arrhythmias make the immediate prognosis grave especially when

quinidine and/or digitalis are exhibited. The most serious types of arrhythmias are frequent ventricular premature systoles and intraventricular block. The presence of congestive heart failure is a grave prognostic sign. There is an added hazard in the use of digitalis in such patients, the immediate mortality being higher in those patients who received digitalis. Thromboembolic phenomena occurring during recent myocardial infarction have a grave prognostic significance.

DISCUSSION

DR. ROY W. SCOTT, Cleveland, Ohio.—Do you have any data on the relation between the duration of shock after the initial attack and the ultimate prognosis? It has seemed to me that the longer the patient remained in shock the surer he was to die.

DR. CECIL STRIKER, Cincinnati, Ohio.—I would like to ask if Dr. Katz has any opinion on the use of anticoagulants as a factor against these mortality statistics.

DR. M. J. SHAPIRO, Minneapolis, Minn.—I should like to ask if Dr. Katz concludes that one should not use quinidine at all in the treatment of coronary thrombosis.

DR. MOSES BARRON, Minneapolis, Minn.—I would like to know how many of the patients showed embolic phenomema. I also would like to ask about the use of digitalis in coronary thrombosis, since Dr. Katz spoke of the dangers of its use. It is true that we have to be careful in the use of digitalis in these cases; however, when there is congestive heart failure digitalis should be used but in a smaller dose. Would this method also contribute to the death in such cases?

DR. HENRY T. RICKETTS, Chicago, Ill.—I should like to ask what evidence Dr. Katz has that the administration of insulin, short of producing hypoglycemia, is harmful to the patient with diabetes with myocardial infarction. If he believes that patients with infarction are benefited by hyperglycemia, is it his custom to administer glucose routinely?

DR. JOHNSON MCGUIRE, Cincinnati, Ohio.—How late in the course of convalescence did embolic phenomena occur?

DR. HAROLD C. LUETH, Omaha, Neb.—I would like to ask Dr. Katz if he made a study of the degree of cyanosis and its relationship to mortality in myocardial infarction.

DR. WARREN B. COOKSEY, Detroit, Mich.—I feel that there is indeed an occasional direct relationship between strenuous exercise and coronary thrombosis. Dr. Katz does not state the time element involved in his study. Surgeons know that several days may elapse before signs of thrombus develop after a blood vessel trauma, and this should be borne in mind in relation to coronary thrombosis. A few years ago a study of 100 patients under my care seemed to show that seventeen of them were sedentary individuals who had engaged in strenuous physical exertion within twenty-four to forty-eight hours of their signs of complete occlusion.

DR. ROY W. SCOTT, Cleveland, Ohio.—What about post-mortem evidence of hemorrhage?

DR. CARL F. SHAFFER, Houston, Texas.—What are your criteria for diagnosis?

DR. KATZ.—We have no adequate data on the duration of shock and the ultimate prognosis. I would consider, however, that the prognosis would be worse the longer the duration of shock.

As to anticoagulants, I think that the cooperative study which Dr. Wright has set up may settle this when 1,000 cases are carefully studied. My own feeling is that a patient who has had a thromboembolic phenomenon, after the original infarct, should receive anticoagulants. I might be persuaded to give it in all instances of recent infarction.

We use quinidine when there is an indication for it. Multiple premature systoles or paroxysmal tachycardia is one indication. However, quinidine is a dangerous drug and should be given cautiously in acute infarction.

Of the 572 cases in our series 107 patients died (21.8 per cent) and fifty-two had thromboembolic phenomena; of these twenty-nine died (55.8 per cent). Thromboembolic phenomena occurred in 10 per cent of all patients. Thromboembolic phenomena occurred in 27.1 per cent of the patients who died.

Of course we give digitalis. If there is an indication give it, give it slowly and carefully. We are not sure what the cause of death is in these digitalized patients; nevertheless, there is sufficient hazard connected with its use to avoid giving it to every patient with an infarct, unless the patient shows progressive congestive heart failure.

As regards insulin and glucose, I made the statement about the danger of giving insulin to patients with angina because such patients then do not do so well. In acute emergencies insulin is covered with glucose.

I have no specific data on when emboli occur during convalescence, but I would say it is within two weeks after the infarction.

We did not study virus infection in the lungs.

About physical exercise, I point to the work of Blumgart that infarction was found to ensue after unduly severe exercise in Army personnel. In our series one patient had a similar story. We admit that when we take a history there are omissions, that patients may not remember all the facts, and that the questions may prejudice the patient.

We have made no analysis of subintimal coronary hemorrhage in this series.

The criteria of location of a lateral wall infarction in the electrocardiogram is published in my book on electrocardiography.

The Michael Reese Hospital patient population is about equally divided between the two sexes, and the male dominance in infarction is therefore not dependent upon an unequal sex hospital population.

PRESENT STATUS OF THE THERAPY OF EXPERIMENTAL RENAL HYPERTENSION*

G. E. Wakerlin, M.D., Oliver Kamm, Ph.D. (By Invitation), Wayne Donaldson, M.S. (By Invitation), W. G. Moss, M.S. (By Invitation), Hiroaki Minatoya, M.S. (By Invitation), Ted Lefco, B.S. (By Invitation), and John Marshall, B.A. (By Invitation), Chicago, Ill., and Detroit, Mich.

The Council has asked me to present a brief review of the recent research of our group at the University of Illinois with reference to the present status of the therapy of experimental hypertension produced by constriction of the renal arteries, and as more or less summarized in three abstracts in this year's Proceedings of the Central Society for Clinical Research.

The three therapeutic approaches which have received most attention in recent years have involved the use of naphthoquinones, marine and fish liver oils, and renal extracts. Several groups of investigators have reported the successful therapy of renal hypertensive rats and dogs with various quinones, especially the vitamin K naphthoquinones. There is at least one recent favorable report in the literature on the use of water-soluble vitamin K in essential hypertension. However, we have found no antihypertensive effect in renal hypertensive dogs from either fat-soluble or water-soluble vitamin K. This, of course, does not preclude effectiveness in human essential hypertension, since the question of a common partial pathogenesis of experimental renal hypertension and essential hypertension is still unsettled.

*Aided by grants from the John and Mary R. Markle Foundation and the Graduate School Research Fund of the University of Illinois.

Following earlier clinical reports of the value of vitamin A concentrate obtained from fish liver oils in essential hypertension, we tested a vitamin A concentrate as well as a number of other vitamins in the treatment of experimental renal hypertension. None of these was effective except the vitamin A concentrate. Since then our group has tested three other lots of presumably the same vitamin A concentrate and found only one to be slightly antihypertensive. Of two other vitamin A concentrates tested, one showed slight antihypertensive activity. Eleven marine and fish liver oil fractions have proved to have no antihypertensive effect. Another research group has likewise found fish liver and marine oils inconstantly to contain variable amounts of an orally effective antihypertensive principle. This principle is definitely not vitamin A. Assay of its value in human hypertension should await further work on the separation, purification, and, if possible, identification of the principle and its production in adequate quantities.

A number of workers have reported the presence of an antihypertensive principle in kidney, but to date no one has produced a consistently effective, reasonably purified preparation properly suited for decisive trial in essential hypertension or human renal hypertensions. Our research group has found a crude renal extract to be highly effective in the treatment of renal hypertensive dogs. At first we suggested that the antihypertensive effect was due to the in vivo neutralization of renin by antirenin produced as a result of the injections of renin contained in the crude extract, but more recent work makes the antirenin hypothesis very doubtful. Since we have obtained evidence that the antihypertensive activity may be in the nonrenin portion of the crude extract, we have studied about thirty fractions of the crude renal extract, some containing little or no renin. Certain of these fractions have proved to be inconstantly active, but we have not as yet been able to produce a consistently active purified preparation. On the basis of our work to date, we have demonstrated the following properties for the antihypertensive renal principle: (1) water-soluble (at least between pH 4.0 to 8.0); (2) retains at least partial potency for one year at pH 4.0 and 4° C.; (3) fairly resistant to slow freezing; (4) partially stable at 70° C. for twenty minutes; (5) poorly resistant to 40° C. for four hours under vacuum at pH 4.0; (6) probably nondialyzable; (7) partially precipitated by $\frac{7}{12}$ saturation with $(NH_4)_2SO_4$; (8) possibly a protein somewhat smaller in size than the pseudoglobulin renin.

We have likewise been able to substantiate our earlier observations that certain renal extracts given prophylactically will prevent the development of experimental renal hypertension in dogs. The technique of renal artery constriction which we have used gives a somewhat higher percentage of hypertension success than that reported by other research groups or obtained by us with slight modifications of the technique.

Recently we have been able to remove renin from the kidney biologically and are in the process of testing nonrenin kidney extract for the antihypertensive renal principle therapeutically and prophylactically. More work is obviously necessary before a sufficiently pure and potent preparation of the antihypertensive renal principle is available for trial in human hypertensions.

DISCUSSION

QUESTION.—What about the fever-producing qualities of these kidney extracts?

DR. WAKERLIN.—We studied this and have found that our renal extracts (which are administered intramuscularly) have no pyrogenic effect.

THE RESULTS OF SURGERY IN PATENT DUCTUS ARTERIOSUS

M. J. SHAPIRO, M.D., MINNEAPOLIS, MINN.

This study was done to determine the effectiveness of surgery in patent ductus arteriosus. An analysis has been made of 643 patients who have been operated upon throughout the world. The differences in results of ligation of the duct as compared to section are determined.

Our figures show that at least 10 per cent of the cases that have been ligated have recanalized. It is also indicated that complications are more common with ligation than with section. The over-all picture is exceedingly good. The total mortality rate is approximately 5 per cent.

An analysis of our own sixty-five cases reveals that we have had the average number of recanalizations in those cases that were ligated. In approximately forty consecutive cases in our group where section has been done, there have been no fatalities. Gross, Wangensteen, and Craaford have done 172 sections of the duct with no fatalities. The follow-up study of the majority of these patients reveals the results as excellent.

It is our conclusion that surgery for patent ductus arteriosus is very effective and that the mortality rate has now been reduced to the point where all children with this lesion should be operated upon whether or not they show any evidence of cardiac strain. Surgery being more hazardous in adults, operation should be done only if such patients reveal evidence of cardiac strain, particularly if this is progressive. Patients with subacute bacterial endarteritis should be operated upon as soon as the diagnosis is made, in most instances, without attempting cure of the infection with chemotherapy. As a result of our study, we are convinced that ligation is ineffective and should be replaced by section of the duct, except in rare instances where section is not feasible.

DISCUSSION

DR. EDWARD MASSIE, St. Louis, Mo.—I would like to ask Dr. Shapiro if he would operate upon a patient with patent ductus arteriosus if another lesion were present. We see that condition frequently and a decision has to be made as to what should be done. I sometimes hesitate in view of the presence of the other lesion.

DR. SHAPIRO.—We had one patient operated upon who had both a patent ductus and an interventricular septal defect. We predicted that the machinery murmur would disappear but that the murmur at the lower end of the sternum would persist after surgery, and this is what happened. It was important in this instance to be aware of both lesions, as it might be concluded that surgery had failed because a murmur in the heart was still present. We have another such case scheduled for surgery. This patient has a patent ductus, an interventricular septal defect, and a complete heart block undoubtedly on the basis of the defective septum. There is every reason why the patent ductus should be closed in such cases.

The question of differential diagnosis between patent ductus arteriosus and interauricular septal defect is not difficult to answer. It must be realized that patency of the duct is an

extracardiac lesion while the interauricular septal defect is intracardiac. A patent duct of any appreciable size is always accompanied by peripheral vascular changes secondary to a wide pulse pressure while an interauricular septal defect of any size produces no change in the peripheral vascular system.

PULMONARY ARTERIOVENOUS FISTULA WITH SECONDARY POLYCYTHEMIA OCCURRING IN TWO BROTHERS: CURE BY PNEUMONECTOMY

ALFRED GOLDMAN, M.D., ST. LOUIS, MO.

This report is based on two cases of pulmonary arteriovenous fistula producing secondary polycythemia, occurring in brothers.

The first case is that of a 22-year-old man who had a long-standing polycythemia ranging up to 11,000,000 red blood cells, cyanosis, and clubbing of the extremities, all of which were known to be present since early childhood. An x-ray of the chest showed a mass in the left hilum and left mid-lung field which on fluoroscopic examination showed pulsation. There was no evidence of heart disease. The diagnosis of a cavernous angioma acting as a pulmonary arteriovenous fistula was made. A left pneumonectomy was recently performed. The lung showed a series of widely communicating channels between the pulmonary arteries and the veins, so that a good portion of the blood entering into the left lung poured directly into the pulmonary veins without oxygenation. Following the operation there was a rapid disappearance of the cyanosis, polycythemia, and clubbing of the extremities.

The second case is that of the 32-year-old brother. The red blood count was 7,000,000. There was a marked cyanosis and slight clubbing of the fingers. An x-ray of the chest showed a mass in the left mid-lung field which had a definite pulsation on fluoroscopic examination.

These two cases are the fourth and tenth cases, respectively, of pulmonary arteriovenous fistula with secondary polycythemia to be reported. This is the first time that the disease has been reported in two members of one family. A study of all ten cases shows such a characteristic and uniform picture that a clinical syndrome due to a large arteriovenous fistula can be established. A combination of polycythemia, clubbing of the extremities, a low arterial oxygen saturation, negative cardiac findings, plus an unexplained chronic pulsating pulmonary lesion is pathognomonic of a pulmonary arteriovenous fistula. Surgical removal of the lesion results in a clinical cure.

DISCUSSION

DR. EMMET BAY, Chicago, Ill.—There was no demonstrable right heart enlargement in the first case. The large pulmonary arteriovenous fistula was shown in the pathologic specimen and I cannot figure out the mechanics of this situation. I would think the output from the right heart would have to be increased with each heartbeat.

DR. MILTON LANDOWNE, Chicago, Ill.—I should like to bring up the following two points:

(1) The tests which are used to support the diagnosis of a right to left shunt in congenital heart disease would also be expected to reveal the presence of a pulmonary arteriovenous fistula. Thus, one would expect peripheral manifestations of intravenously injected

ether (as described for congenital heart disease by Benenson and Hitzig) and possibly an apparent reduction in the "arm to tongue" depending on the size of the shunt and the amount of material used for the test.

(2) Adams, Thornton, and Eichelberger recently have reported upon the successful surgical treatment of this condition. In their case a familial nature of the disease was also suggested. They found a suggestive pulmonary shadow in the x-ray of the patient's father and in addition, their patient, his father, and possibly several other members of the family had bleeding telangiectases of the nose and mouth. I wonder whether these cases may not ultimately be classified, therefore, as a variety of Osler's familial hemorrhagic telangiectasia.

DR. GOLDMAN.—One would expect to find enlargement of the right heart as the result of a pulmonary arteriovenous fistula. However, it did not occur; this is in contrast to the situation in peripheral arteriovenous fistulas. There has been no case report in which there are any changes in the heart as a result of the fistula. This is explained by the fact that the lesion involves only the pulmonary circulation. None of the three cases that came to post mortem showed any cardiac changes attributable to the arteriovenous aneurysm.

THE DIAGNOSTIC VALUE OF ELECTROCARDIOGRAPHIC PATTERNS BASED ON AN ASSAY OF 261 ADDITIONAL AUTOPSIED CASES

L. N. KATZ, M.D., D. FELDMAN, M.D. (BY INVITATION), AND
R. LANGENDORF, M.D., CHICAGO, ILL.

An analysis was made of a consecutive series of 261 cases necropsied within two months after they had had an electrocardiogram made. The purpose of this study was to determine the accuracy and dependability of our electrocardiographic criteria. The thirteen specific patterns encountered are enumerated and the criteria listed. In this series forty-one cases were encountered in which discrepancy existed between the necropsy findings and the electrocardiogram; in the other 220 cases (84.3 per cent) no discrepancy was found. In general the electrocardiogram is a good index of anatomic normality and abnormality of the heart, although its use is limited by its inability to distinguish in many cases between the clinical and subclinical forms of heart disease. An abnormal electrocardiogram is excellent evidence for anatomic abnormality. A normal electrocardiogram, however, is occasionally found with an abnormal heart.

Ventricular hypertrophy is usually reflected in the electrocardiogram by heart strain patterns. Left ventricular hypertrophy more often gives rise to its strain pattern than right ventricular hypertrophy. With combined ventricular hypertrophy the pattern seen is of the predominantly hypertrophic ventricle more often than a pattern diagnostic of combined heart strain.

Recent myocardial infarctions, especially if serial records are available, are seldom missed electrocardiographically. However, old infarcts or severe coronary sclerosis without confluent infarction sometimes give rise to patterns resembling those of recent infarction. Old healed myocardial infarctions can often be diagnosed electrocardiographically but not with the regularity of recent infarction. Coronary sclerosis without myocardial infarction generally results in nonspecific electrocardiographic abnormalities.

This study confirms our previous one, making a total of 410 consecutive cases analyzed. The value of specific patterns in electrocardiography, when properly used, is thus established.

ANTITULARENSE SERUM: CORRELATION BETWEEN PROTECTIVE CAPACITY FOR WHITE RATS AND PRECIPITABLE ANTIBODY CONTENT

LEE FOSHAY, M.D., I. RUCHMAN, PH.D. (BY INVITATION), AND P. S. NICHOLES, PH.D. (BY INVITATION), CINCINNATI, OHIO

Antitularense sera have hitherto failed to reveal protective antibody against challenge with strains of maximal virulence whenever the test animals were the mouse, guinea pig, hamster, or rabbit. Past inability to protect highly susceptible animals has encouraged an unfounded belief that serum therapy is therefore ineffective in the human disease. Since the reaction to tularemic infection by the rat approximates more closely that of man than do those of other animals, the rat was used for serum protection experiments.

Protective antibody was readily demonstrated in 2 c.c. quantities of each of four antitularense sera by intraperitoneal injection of the 85 to 110 gram white rat and by immediate subcutaneous challenge with 25 million viable organisms of maximal virulence, an average of 25,000 LD_{50} rat doses.

Precipitable antibody content of sera was quantitated by the Culbertson neutralization method, using a polysaccharide prepared from acetone-extracted cells of a virulent strain by the phenol extraction method of Palmer and Gerlough.

Rat mortality was the criterion by which serum protection was judged.

Prechallenge treatment with normal sera gave 95 per cent mortality. Pretreatment with the most potent hyperimmune serum, aged fifteen months, gave 12.5 per cent mortality. Sera of lesser potency gave correspondingly greater mortality. Mortality rates were roughly inversely proportional to precipitable antibody contents of sera. There was good agreement between antibody content and protective capacity for four immune sera of graded potencies.

The difference in clinical effectiveness between the two best sera was of the order of that shown by both rat protection and antibody content tests.

INFLUENZA B: STUDY OF A LOCALIZED OUTBREAK PRECEDING THE 1945 EPIDEMIC*

A. E. FELLER, M.D., CLEVELAND, OHIO

The present report is concerned with a clinical and serologic study of a localized outbreak of influenza B, which occurred in a battery of 230 recruits during the summer of 1945 at Fort Bragg, N. C. The battery had been under observation previous to the outbreak and was studied for a total period of approximately four months. Blood specimens were obtained at the beginning and end of the period of observation, thus providing the basis for correlating the clinical studies with the serologic findings.

The data showed that "recognized" and "inapparent" infections occurred with approximately equal frequency. The "recognized" infections occurred principally during a period of three weeks, indicating that a definite outbreak had occurred. However, more than half of the total infections were concentrated

*From the Commission on Acute Respiratory Diseases.

in only one of the four platoons comprising the battery. Thus, the outbreak was localized to one segment of the population, and only sporadic infections occurred in the remainder. There was a direct but not complete relationship between low antibody titers of the initial sera and subsequent large increases in titer to influenza virus B. Also, the initial sera from those individuals who subsequently developed a febrile respiratory illness tended to have lower titers than did the sera from individuals who had either an afebrile respiratory illness or no recognized illness.

The localized nature of the outbreak seems to have been characteristic of the manner in which influenza B occurred in other parts of the country during the period of several months preceding the widespread epidemic of influenza B in the fall and winter of 1945. The fact that influenza A also occurred in a similar manner during a period of several months preceding the 1943 epidemic suggests that the sporadic or localized occurrence of influenza may indicate the imminence of an epidemic. Furthermore, the correlations between initial antibody titers and the subsequent development of serologic or clinical evidence of infection indicated that these immunologic aspects of influenza B are similar to those of influenza A.

EVALUATION OF THE TREATMENT OF PRIMARY PNEUMOCOCCIC PNEUMONIA

ROBERT T. THOMPSON, M.D. (BY INVITATION), AND
M. A. BLANKENHORN, M.D., CINCINNATI, OHIO

The degree to which penicillin should supplement sulfonamide drugs in the treatment of pneumococcic pneumonia is a question at the present time. Rather diverse results have been reported from various clinics so that evaluation of results is difficult. One reputable 1945 publication gives two consecutive abstracts on the treatment of pneumococcic pneumonia in which fatality rates are 18.5 and 1.1 per cent, respectively. The treatment of 189 cases of primary pneumococcic pneumonia is evaluated here in an effort to resolve apparent discrepancies in the comparison of other reports.

All of these patients were treated with sulfamerazine. A few received antipneumococcic serum, and a few of the later patients were given penicillin after complication developed.

Of the 189 patients treated twenty-six died. Of the 122 patients over 40 years of age twenty-four died. Of twenty-eight patients with bacteremia fourteen died. Type VII pneumococcic pneumonia occurred in thirty-five patients, and eight of these died. Of eight patients who had type VII pneumococcus bacteremia seven died.

Elevation of the blood urea nitrogen above 40 mg. per cent was found in thirty-three of the 189 patients treated. Of these thirty-three patients fourteen recovered and nineteen died.

Nineteen of the twenty-six fatal cases had azotemia prior to death. In fourteen of these it was as great, or greater, before sulfamerazine was started than at any subsequent time. Three others had azotemia before treatment which increased progressively until death. Another had mild azotemia while in shock

prior to death, and the last had a necrotizing angiitis due to sulfonamide hypersensitivity which involved the kidneys, heart, liver, and spleen.

Eleven of the sixteen autopsied cases showed pathologic abnormality of the kidneys. One had hypersensitivity angiitis of kidneys, two had toxic nephrosis, and eight had chronic disease of the kidneys.

Review of the fatal cases showed that shock, cardiac arrhythmia, and pulmonary edema were notable in the death of pneumococcic pneumonia patients. Every one of the sixteen autopsied cases showed pathologic abnormality of the heart; in one there was thrombotic nonbacterial endocarditis; in four there were changes of an acute toxic nature; and in eleven there were chronic or degenerative changes.

Seven patients who died were treated less than twenty-four hours.

It is concluded that delay in treatment and aggravation of latent disorder often defeat specific treatment for pneumococcic pneumonia.

DISCUSSION

DR. A. E. FELLER, Cleveland, Ohio.—It has been shown that pneumococcic pneumonia can be prevented, but the statement has been made that we now have such good results with sulfonamides and penicillin that it is not worth while to immunize people against this type of pneumonia. It is true that pneumococcic pneumonia does not have a high death rate or high incidence rate in certain age groups, but it seems to me that it would still be worth while to immunize against it because in many circumstances people still die from pneumococcic pneumonia as this study so clearly emphasizes.

A STUDY OF THE ACTION OF BETA-DIMETHYLAMINOETHYL BENZHYDRYL ETHER HYDROCHLORIDE (BENADRYL) IN THE SKIN OF HUMAN BEINGS

MILO D. LEAVITT, JR., M.D. (BY INVITATION), AND CHARLES F. CODE, M.D., ROCHESTER, MINN.

A study has been made of the mode of action, in the skin, of beta-dimethylaminoethyl benzhydryl ether hydrochloride (benadryl), a new synthetic antihistamine compound. The experiments were performed on the skin of thirty-three healthy, nonallergic persons; the size of histamine flares resulting from the standard intracutaneous injection of 1 microgram of histamine base was utilized as an indicator of the effectiveness of benadryl as an antihistamine agent. From photographic records made during each procedure, the areas of flares were accurately determined by the use of a planimeter.

Control studies performed on each person revealed that maximal flaring caused by histamine injected into skin infiltrated with isotonic solution of sodium chloride occurred during the first fifteen minutes in 90 to 95 per cent of the persons. During this time interval the maximal size of the flare in forty experiments varied from 16.1 to 24.7 square centimeters.

Histamine flares superimposed on skin infiltrated with benadryl diluted 1:1,000 were reduced 61.6 per cent in size, as compared with control flares. The intravenous administration of 150 mg. of benadryl to ten persons resulted in a 51.7 per cent reduction in size of the flare. The oral administration of 150 mg. of benadryl reduced the size of the flare only 33 per cent.

Benadryl was found to produce local anesthesia when it was injected into skin. Bio-assay, by means of electric algesimetric determinations, showed that benadryl in dilutions of 1:500, 1:1,000, 1:5,000, 1:10,000, and 1:20,000 possesses anesthetic potency similar to that of dilutions of procaine hydrochloride of 0.5, 0.25, 0.125, 0.0625, and 0.03125 per cent, respectively. Equivalent anesthetic doses of benadryl and procaine hydrochloride exerted definite, but not equal, antihistamine action. Procaine hydrochloride in dilutions of 0.0625 and 0.03125 per cent reduced the size of flares only 23.6 and 23.3 per cent, respectively, whereas benadryl of equal anesthetic potency, 1:10,000 and 1:20,000, caused an 80 per cent reduction in the size of histamine flares.

It was found that the pronounced antihistamine activity of benadryl is not dependent on its anesthetic potency. This was demonstrated in experiments in which injections of histamine were delayed until the anesthetic effects of benadryl and procaine hydrochloride had subsided. Flares developing in sites in which procaine hydrochloride was no longer exerting its anesthetic effect closely approximated the size of control flares. Benadryl, however, in all dilutions reduced the size of flares from 86 to 91 per cent, despite the disappearance of its anesthetic effect.

DISCUSSION

QUESTION.—Have you had any experience with pyribenzamine?

DR. LEAVITT.—Our preliminary experience would lead us to believe its local skin effects are very similar.

DR. CODE.—Unfortunately, even though benadryl is so potent a local anesthetic, it has irritant qualities. Other local anesthetic agents may possess antihistamine properties. State and Wangensteen used procaine in the treatment of delayed serum sickness (J. A. M. A. 130: 990-995, April, 1946). It is to be hoped that continued work with these and other antihistamine drugs will aid in solving some of the perplexing problems pertaining to histamine metabolism.

THE OCCURRENCE OF COMBINED SYSTEM DISEASE IN PERSONS WITH PERNICIOUS ANEMIA DURING TREATMENT WITH THE LACTOBACILLUS CASEI FACTOR (FOLIC ACID)

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Remissions in the macrocytic anemia of pernicious anemia, sprue, and pregnancy have been produced repeatedly with the *Lactobacillus casei* factor. This study was undertaken in November, 1945, to answer the following questions: (1) Will *L. casei* factor control the anemia of pernicious anemia and sprue for long periods of time as successfully as liver extract? (2) Will *L. casei* factor therapy prevent the development of the neurologic manifestations of pernicious anemia or control them after they have appeared? This paper presents a progress report at the end of the first year of the study.

The twenty-six subjects of the investigation were patients from the medical wards and dispensary of the Cincinnati General Hospital, twenty-one of whom had pernicious anemia which had been controlled for two to seventeen years by injections of liver extract. Three had pernicious anemia in relapse, and two had sprue which had been poorly controlled by liver extract. Each of these subjects

was treated with 70 to 105 mg. *L. casei* factor per week in divided doses given orally for ten to twelve months. All liver therapy was discontinued. Complete physical and neurologic examinations, erythrocyte counts, hemoglobin levels, hematocrit determinations, and reticulocyte counts were performed at the beginning of the study and were repeated as often as the condition of the patient demanded.

Most of the patients noted an increase in appetite and weight during the year. In only two instances were there moderate decreases in the hematologic values; these occurred after five to eight months while the subjects were receiving the same amount of *L. casei* factor which in each instance had produced a satisfactory remission previously. All manifestations of sprue were well controlled.

After five to eight months parasthesias in the extremities and an unsteady gait developed in four subjects with pernicious anemia. Two of these four subjects are mentioned previously as having developed moderate reductions in hematologic values. Neurologic signs gradually appeared and increased in numbers until there was unequivocal evidence of combined system disease. At this point each of these four patients received 50 to 500 mg. *L. casei* factor daily by mouth for ten to forty days without subjective or objective improvement. Refined liver extract, 5 c.c. intramuscularly daily, was begun after it was obvious that neurologic improvements would not occur or when progression of the disease was so rapid as to endanger the patient's future well-being. Within ten days thereafter subjective and objective neurologic improvement occurred and convalescence is progressing satisfactorily.

The conclusions are drawn that oral *L. casei* factor, 70 to 105 mg. in divided doses per week, will usually maintain patients with pernicious anemia and sprue in a normal hematologic state for a year but will not prevent the occurrence of degenerative disease of the spinal cord and peripheral nerves in patients with pernicious anemia.

NONUTILIZATION OF CONJUGATED FOLIC ACID IN PERNICIOUS ANEMIA

ROBERT W. HEINLE, M.D., EVELYN M. NELSON, B.S. (BY INVITATION), H. VICTOR NELSON, B.S. (BY INVITATION), AND ARNOLD D. WELCH, PH. D., M.D. (BY INVITATION), CLEVELAND, OHIO

The response of pernicious and allied macrocytic anemias to folic (pteroylglutamic) acid implies that a vitamin deficiency is corrected by this therapy. Of the folic acid occurring in foods, a large proportion consists of conjugates of higher molecular weight; thus, the conjugate of yeast (heptaglutamate) contains six additional glutamic acid molecules. Most mammalian tissues contain enzymes and conjugases that convert heptaglutamate to folic acid, and various animal species effectively utilize this conjugate in lieu of folic acid. Parenteral administration of heptaglutamate to normal man markedly augments the urinary excretion of the free vitamin.

In three patients with pernicious anemia studied, no hematologic response followed either oral or parenteral administration of the conjugate, but a definite response occurred in two of these to whom an equivalent amount of folic acid

was given subsequently. Furthermore, in contrast to the finding in normal man, administration of heptaglutamate to the three patients studied caused little or no augmentation of the urinary elimination of folic acid. Bethell and associates have independently observed the ineffectiveness of heptaglutamate in several patients with pernicious anemia.

Although these findings may offer a basis for the apparent folic acid deficiency of pernicious anemia, a conclusion that the utilization of heptaglutamate is due to a direct action of the antipernicious anemia factor is at present unjustified. Augmentation of the conjugase activity of human bone marrow in vitro has appeared to result from the addition of highly purified liver extract, but the results are obtained inconsistently and are of uncertain significance. Conceivably, liver extracts contain a factor necessary for the proper functioning of a conjugase system or a substance concerned with the removal of an inhibitor of a conjugase system. However, the ineffective utilization of the naturally occurring conjugate by patients with pernicious anemia may be secondary to a more deep-seated biochemical derangement.

In favor of this view is the observation that the activity, in patients with pernicious anemia of those conjugase systems studied, is not significantly less than that of normal subjects. Also, the striking effectiveness of purified liver extract in one patient in whom folic acid was almost ineffective, in intramuscular doses up to 12 mg. daily, suggests that the factor(s) of purified liver extracts, although apparently concerned in some way with the release of folic acid from its conjugate, in addition has other functions.

FURTHER STUDIES ON THE UTILIZATION OF PTEROYL HEXAGLUTAMYL GLUTAMIC ACID (VITAMIN B_c CONJUGATE) IN PERNICIOUS ANEMIA

FRANK H. BETHELL, M.D., ANN ARBOR, MICH., MARION E. SWENDSEID, PH.D. (BY INVITATION), ORSON D. BIRD, PH.D. (BY INVITATION), AND RAYMOND A. BROWN, PH.D. (BY INVITATION), DETROIT, MICH., MURIEL C. MEYERS, M.D., AND GOULD A. ANDREWS, M.D., ANN ARBOR, MICH.

The effectiveness of pteroylglutamic acid* (P. G. A.) in the treatment of certain macrocytic anemias raises the question of the cause of a deficiency of this substance in such anemias. Although widely distributed in common foods, this component of the vitamin B complex is usually present as a conjugate, pteroyl hexaglutamyl glutamic acid. Consequently an apparent deficiency of P. G. A. in persons whose diets have not been grossly inadequate suggests a metabolic defect in the utilization of the natural material. Evidence has been obtained of such a defect by observations of hematologic responses and by determinations of the urinary excretion of P. G. A. in patients with anemia after the oral administration of the vitamin in the conjugated and in the free form. In this communication the results of earlier studies as well as additional investigations on this problem will be reported.

The hexaglutamyl conjugate was given orally in the form of a yeast concentrate to fourteen patients and to six healthy subjects. There were nine

*This is the chemical name of the vitamin which has been referred to as folic acid, vitamin M, or vitamin B_c.

patients with pernicious anemia in relapse, three with pernicious anemia in liver extract induced remission, and two with macrocytic anemia following total or subtotal gastrectomy. Doses of conjugate supplying the equivalent of 4 to 5 mg. of crystalline P. G. A. daily were without effect on the erythrocytes, reticulocytes, hemoglobin, or hematocrit values of any of the patients with anemia. The administration of equal amounts of the vitamin in the free form (Folvite*) was followed in all instances by significant hematologic increases, usually approximating the expected results from adequate oral liver extract or stomach therapy.

The P. G. A. content of the urine was determined by microbiologic assay. No evidence for the excretion of the vitamin as conjugate was obtained in any of the experiments. The excretion of the vitamin while the subjects received unsupplemented diets was generally lower in the case of the patients with macrocytic anemia than in the case of the normal persons.

After the administration of the vitamin conjugate the patients with macrocytic anemia showed no significant increase in excretion of P. G. A., with the exception of one subject who excreted slightly greater amounts, whereas the normal individuals excreted relatively large quantities. After the administration of free vitamin the patients excreted variable amounts, whereas the values for the normal subjects were considerably higher and more constant.

Administration of conjugate to three patients with pernicious anemia who had been receiving parenteral liver extract for a number of years and whose blood values were within normal limits was followed by excretion of the free vitamin in amounts comparable to those excreted by normal persons.

It is believed that these results demonstrate that the normal person can convert the vitamin conjugate to the free form and that in untreated pernicious anemia this ability may be either greatly reduced or entirely lacking.

It seems probable that for P. G. A. to participate in hematopoiesis (megaloblastic maturation) the vitamin must be present in the free state.

The difference in excretion levels of the vitamin after administration of the conjugate, between patients in relapse and those in liver extract-induced remission, suggests that the principle of liver active in pernicious anemia may be concerned with the conversion of the conjugated vitamin to the free form.

DISCUSSION

DR. BYRON E. HALL, Rochester, Minn.—During the past year observations similar to those just reported by Dr. Vilter and his associates have been made at the Mayo Clinic; namely, that administration of synthetic folic acid neither protects the patient with pernicious anemia from neurologic manifestations nor relieves him of them once they have developed. In our series of fourteen patients with pernicious anemia who were treated for many months by administration of synthetic folic acid, ten instances of peripheral neuropathy as manifested by paresthesia and two of early subacute combined degeneration of the spinal cord were observed before institution of treatment. Of the ten instances of paresthesia improvement occurred in four, temporary improvement followed by relapse occurred in four, and no improvement was observed in two. In the two instances of subacute combined degeneration of the spinal cord which were observed before institution of treatment, evidence of progression of the neurologic manifestations of the disease was noted during administration of synthetic folic acid. In the cases in which symptoms or signs of neurologic involvement did not exist prior to the institution of treatment with folic acid, two instances of paresthesia and three of early

*Lederle Laboratories, Inc., Pearl River, N. Y.

signs of combined sclerosis occurred while treatment was being given. Neurologic manifestations developed in some cases despite induction of satisfactory hematologic remissions.

Another observation has been that of the wide variation in rapidity of hematologic response of patients with pernicious anemia in relapse who were treated with synthetic folic acid. While an occasional patient responds as rapidly to treatment with folic acid as to treatment with liver, the majority respond hematologically more slowly to treatment with folic acid. The response of three of fourteen patients treated with folic acid was very slow and hematologic remissions could not be induced despite administration of fairly large doses of the preparation; however, satisfactory hematologic remissions were induced in all three patients by means of intensive treatment with liver.

The appearance of sternal marrow obtained by serial aspiration in our group of cases has indicated that synthetic folic acid rapidly converts erythropoiesis from a megaloblastic to a normoblastic type. While the effect of this agent on the bone marrow and blood of patients with pernicious anemia in relapse is of great interest, it is obvious that the substitution of synthetic folic acid for liver in treatment of pernicious anemia is extremely hazardous in view of the demonstration that administration of folic acid does not prevent development of neurologic manifestations of the disease.

DR. WALTER L. PALMER, CHICAGO, ILL.—The first two papers and the discussion by Dr. Hall all suggest that folic acid is not a complete substitute for liver extract. It may well be that this is true, but we should be very careful in arriving at this conclusion. I am reminded of the experience we all have had with liver through the years. At first we thought it was not satisfactory in all cases; then we thought the crude form was better than the refined; and finally we decided that refined liver extract was a complete substitute for whole liver and for the crude extract as well. The problem seemed to resolve itself into (1) the method of administration and (2) the dosage. In the first paper the route of administration of the folic acid was oral. We all know that the oral method of administration of liver extract is very unsatisfactory in many instances and that there are some patients who cannot be maintained with normal blood pictures in this manner. With regard to dosage, apparently the largest amount of folic acid given intramuscularly was 12 milligrams. This may not be enough. Would it not be worth while to try 50 mg. per day intramuscularly?

DR. CECIL J. WATSON, Minneapolis, Minn.—I want to ask whether in the urinary excretion studies the results were based on bio-assay technique and whether any studies on conjugated or free folic acid have been made on the feces of these patients.

DR. HOWARD L. ALT, Chicago, Ill.—I would like to ask about the diet these patients received who had relapses of cord disease on folic acid treatment.

DR. M. W. COMFORT, Rochester, Minn.—It might be worth while at this time to mention the disappointing results obtained so far in the treatment of nontropical sprue with synthetic folic acid. This is in contrast to the excellent response of tropical sprue to treatment with the same preparation. My colleagues and I have treated a number of patients who have nontropical sprue with adequate doses of synthetic folic acid for several months. Four patients received 50 to 200 mg. of folic acid a day intramuscularly, and the results to date are as follows: Diarrhea has not been significantly affected and relapses have continued to occur with about the same degree of frequency. In one patient in whom megaloblastic bone marrow was present the bone marrow changed rapidly to a normoblastic type. In all four patients the reticulocytes have responded slightly, but so far the percentage has not exceeded 4. In none of these patients has the size of the erythrocytes been reduced or has the anemia improved to a satisfactory degree. One patient is dead apparently as the result of an exacerbation of the disease, and two are in exceedingly serious condition. Of the patients given folic acid by mouth, changes have not been seen in their condition that could not be ascribed to the fluctuations in the severity of the disease. These disappointing results suggest that in some patients nontropical sprue will not respond favorably to treatment with synthetic folic acid.

DR. R. W. VILTER, Cincinnati, Ohio.—Regarding Dr. Palmer's comments, in working up this paper for presentation I went back over the experiences from 1929 to 1935 with liver extract in this same type of patient. There was considerable divergence of opinion, as

Dr. Palmer indicated, as to the effectiveness of liver extract in combined system disease of pernicious anemia. From the literature I was unable to tell why there was this divergence of opinion because most of the liver extract was given intramuscularly in adequate doses, so I could not come up with any analogy between their experience at that time and our experience now with folic acid. Generally speaking folic acid given by mouth is almost as effectively absorbed and utilized as folic acid given intramuscularly. The average effective dose is usually 2 to 5 mg. by the parenteral route or 10 to 15 mg. by oral route. In contrast, liver extract is poorly absorbed and utilized by the oral route, the effective oral dose being many hundred times the intramuscular dose. The interesting thing regarding the diets of the patients who relapsed is that patients whom we knew were on deficient diets did not develop neurologic symptoms, whereas patients in the private group who were referred because of sensitivity to liver developed neurologic manifestations. The diets of the patients in the private group were much better than those of the patients from the dispensary. I do not believe that diet had much to do with these relapses.

As to Dr. Comfort's comments, I have a feeling that nontropical sprue is a group of diseases having poor intestinal absorption and steatorrhea as a common denominator rather than a single disease. Folic acid will not benefit patients who appear to have sprue but in reality have some other disease, for instance lipophagic granulomatosis. The two patients I mentioned were from Cincinnati and would be classified as nontropical sprue; they both fared better on folic acid than they had in five years on liver extract.

Dr. Robert W. Heinle, Cleveland, Ohio.—We have forty patients with pernicious anemia who have been under treatment with folic acid for from seven to nine months. They had received liver extract for many months or years before folic acid was given. In the short period they have been on folic acid, none of them has shown neurologic relapse. I do not want you to think that I am overenthusiastic about this drug. I do not think it has been proved to be as good as liver extract, but I do agree with Dr. Palmer that we have not tested it out thoroughly, particularly as to the route of administration and the dosage.

Dr. Palmer criticized the case of the one patient to whom we gave 12 mg. daily. His is a valid criticism, but that patient had been on an experimental regimen for fifty-two days without benefit. Other patients on that dose had received more benefit. Whether this one patient would have received more benefit if the folic acid had been given in larger doses I do not know.

The method of assaying conjugated folic acid in the urine is the standard method utilizing *Lactobacillus casei*. We have attempted to make these same studies on feces. This is difficult because of the high bacterial content. We are working on that problem at present and our preliminary study would indicate that very little folic acid is present in the stool.

With reference to Dr. Comfort's remarks about nontropical sprue, I have had a limited experience. Tremendous doses of liver seem to be necessary to obtain satisfactory results in many cases. Intravenous administration of crude liver extract has been recommended. The feeling has been that intravenous doses of crude liver extract in large amounts may be better than the administration of purified liver extract.

There is one thing I would like to say about the clinical use of folic acid. The dose schedule recommended by the manufacturer is too low. They recommend daily doses of 5 to 20 mg. or intramuscular doses of 15 mg. daily. It is possible that some patients can be brought into remission on that dose, but it is more likely that a great many will need larger doses than this, and I do not think we should pay too much attention to the recommended schedule. It should be noted that the manufacturers of folic acid suggest that better results may be obtained by the simultaneous administration of folic acid and liver extract than with folic acid alone.

RUTIN IN PURPURA

F. W. Madison, M.D., and H. W. Pohle, M.D. (By Invitation),
Milwaukee, Wis.

It is probable that one of the etiologic mechanisms capable of producing the vascular fragility characteristic of purpura is of deficiency type. For many

years avitaminosis C was considered to be of major importance, but recent studies have suggested that it is much less frequently a factor than was previously supposed. In 1936 Szent-Györgi observed that citrin derived from paprika or lemon was capable of reversing experimentally produced vascular fragility. He designated it as vitamin P and suggested that deficiency of that substance was responsible for purpuric changes in some instances. Two crystalline flavone glucosides derived from citrin, hesperidin, and eriodyctol have failed to produce the same effect noted in the use of citrin. Recently a flavonol glucoside, rutin, closely related to hesperidin, has been used in a similar manner and has seemed to be more effective than either of the flavone glucosides. It has been used in 173 reported cases of purpura with reversal of fragility in 152 cases (88 per cent).

Our clinical experience with rutin has been limited by the supply available, but the results thus far have been of such nature as to suggest that it does exert a favorable influence on vascular fragility in some types of purpura. Its effect has been studied in fourteen cases of clinical purpura, six of which were of probable allergic origin, two toxic, one endocrine, one associated with hypertension and retinal hemorrhages, and four associated with advanced malignant disease. The most striking results were obtained in the last group where cessation of the drug resulted in relapse and resumption in remission. Less convincing results were seen in the allergic group and questionable results in the remaining groups.

THE EFFECT OF TOTAL BODY X-IRRADIATION ON A PRE-EXISTING INDUCED ANEMIA

LEON O. JACOBSON, M.D., EDNA K. MARKS (BY INVITATION), AND ERIC L. SIMMONS, PH.D. (BY INVITATION), CHICAGO, ILL.

One of the problems facing those of us charged with the responsibility of the protection of personnel from radiation hazards on the plutonium project was whether individuals with a pre-existing anemia would be more adversely affected by externally originating total body irradiation than individuals with normal values. In an attempt to study this problem, a regenerative anemia was produced in rabbits by the subcutaneous administration of phenylhydrazine hydrochloride. The hematologic effects of 800 r. total body x-irradiation on such anemic animals were compared to effects on animals given only phenylhydrazine and allowed to recover spontaneously and to a group of animals given 800 r. alone. A group of normal untreated animals was studied simultaneously.

The characteristic initial rise and subsequent reduction in heterophils and immediate precipitous fall in the lymphocyte values followed the administration of 800 r. in the group in which a regenerative anemia had been produced with phenylhydrazine as well as in normal control animals. The time of recovery of those elements to normal limits in the two groups was likewise comparable. An "abortive rise" in the leucocyte values of both of these groups was noted between the fifth and eleventh days after the irradiation.

The group of animals which received x-irradiation alone developed an anemia with its maximum at fourteen days and with recovery by twenty-three days. The animals in which a regenerative anemia was produced by phenyl-

hydrazine and which were irradiated at or near the point of maximum anemia developed no further anemia, values returning to normal limits by twenty-three days. The erythrocyte and hemoglobin values of the group in which an anemia was produced by phenylhydrazine and which were allowed to recover spontaneously, however, returned to normal within fourteen days.

The reticulocyte values of animals which received phenylhydrazine had reached approximately 21 per cent when the x-irradiation was given and were reduced to a minimum value of 1.0 per cent within two days after the irradiation. Recovery to normal limits occurred by nine days in this group. The reticulocyte values of normal control animals which were given 800 r. fell maximally within two days, reached a minimum of less than 0.1 per cent, and recovered slowly but completely by twenty-three days.

The failure of 800 r. total body x-irradiation to produce further anemia in animals with a previously induced regenerative anemia or to materially interfere with recovery is dependent in part on the number of surviving erythroblasts in the erythropoietic tissue.

Histologic studies made on animals prepared in a comparable manner and sacrificed at varying appropriate intervals indicate that erythropoietic tissue was completely destroyed by seventy-two hours in the normal rabbits which received 800 r. and recovery proceeded slowly. On the other hand, although massive destruction of erythropoietic tissue occurred after exposure to 800 r. in animals in whom a regenerative anemia had been produced with phenylhydrazine, this destruction was delayed beyond seventy-two hours. This appears to indicate that a latency to x-ray effect was produced in erythropoietic tissue which had previously been stimulated.

ON THE RELATION OF CALCIUM TO THE ACTIVATION OF PROTHROMBIN AND ITS SIGNIFICANCE IN DICUMAROL POISONING

ARMAND J. QUICK, M.D., MILWAUKEE, WIS.

The role of calcium in the coagulation mechanism has not been satisfactorily defined, and the generally accepted concept that prothrombin is activated by thromboplastin in the presence of ionic calcium has failed to advance knowledge concerning the chemical action of calcium in its participation in coagulation. The recent finding (Steinberg) that a phenol-formaldehyde resin known commercially as Amberlite can quantitatively remove calcium from blood, without producing any other demonstrable change, offers a new means for studying quantitatively the calcium requirement for coagulation and specifically for the activation of prothrombin.

It was found that a fixed amount of calcium is required for the optimum activation of prothrombin, that is, for the shortest prothrombin time. For human plasma it is approximately 0.0012 M. and for dog blood 0.0004 M. Higher concentrations have no further effect except that a depressive action begins when the calcium ion concentration exceeds 0.02 M. Below the optimum or critical concentration of calcium, the prothrombin time becomes progressively delayed. Since this critical level is below the concentration of the

free or ionized calcium, it appears that ionized calcium is not involved in coagulation. One can conjecture that plasma contains a calcium cofactor which with thromboplastin and calcium becomes the activator of prothrombin. Evidence was obtained that this cofactor is not directly dependent upon either component A or B of prothrombin.

When Dicumarol was fed to dogs, the minimum or critical concentration of calcium required for optimum activation of prothrombin increased as the prothrombin (or more accurately component B) decreased. When the prothrombin time became twenty seconds, the normal concentration of the calcium of the blood was no longer sufficient for optimum prothrombin activity. This suggests that the delayed coagulation in dicumarol poisoning may not be solely the resultant of prothrombin depletion but may be influenced by an inadequate concentration of calcium. On the basis of these results, the unreliability of the bedside method for the determination of prothrombin becomes obvious, since in this test thromboplastin without additional calcium is added directly to blood.

DISCUSSION

DR. CHARLES F. CODE, *Rochester, Minn.*—I should like to ask if Dr. Quick has tested the effect of changes in the concentration of prothrombin upon the optimum calcium concentration in an artificial setup using purified constituents in which the prothrombin concentration might be varied at will. I wonder if a shift in the amount of prothrombin alone would alter the optimum calcium concentration.

DR. KENNETH M. BRINKHOUS, Iowa City, Iowa.—Since amberlite is very effective in removing electrolytes, I wonder if you have data on the concentration of other plasma electrolytes in the amberlite-treated plasma. This factor might have had an effect on some of these results, as electrolyte concentration is known to influence considerably the rates of several of the clotting reactions.

DR. QUICK.—In regard to Dr. Code's question about an artificial setup, we have had no experience. This work is still preliminary and we need to do much more on it. Probably we can set up certain experiments to get further information.

In answer to Dr. Brinkhous' remarks, we use amberlite treated with saline and so far as we know the action is primarily an exchange one, an atom of sodium for one of calcium. It gives off one atom of sodium and takes up one of calcium. There may be other changes, and probably the action is not as simple as that, but in our work we found no evidence of action other than the one of exchange.

MORPHOLOGY OF BONE MARROW

IMPRINT AND HISTOLOGIC PREPARATION OF ASPIRATED STERNAL MARROW UNITS

E. M. SCHLEICHER (BY INVITATION), AND E. A. SHARP, M.D., DETROIT, MICH.

It has been established that direct and indirect (heparinized and centrifuged) bone marrow spreads and differential counts made within a measured area offer a means of distinguishing morphologic differences as well as quantitative relationships.

At the same time these methods fail in two important features, namely, (1) the preservation of cellular cyncytia which permit a study of marrow cell development and (2) maintaining intact the histologic pattern of sternal marrow units. These latter configurations are essential in the diagnosis of early infectious or malignant processes of this hemopoietic organ.

It is the purpose of this paper to present representative examples of "imprint" and intact "histologic" preparations of sternal marrow in black and white and kodachrome, showing the methods of preparation and the normal compared with the pathologic morphology. It will be demonstrated that maturation arrest in pernicious anemia does not occur at the megaloblastic (pronormoblastic) stage but rather that the reticulum is pathologically activated. Metastatic carcinoma, tuberculosis, and various patterns in blood disorders will be presented.

THE EFFECT OF RADIATION THERAPY ON THE NOCTURNAL GASTRIC SECRETION IN PATIENTS WITH DUODENAL ULCER

ERWIN LEVIN, M.D. (BY INVITATION), WALTER LINCOLN PALMER, M.D., AND JOSEPH B. KIRSNER, M.D., CHICAGO, ILL.

The fasting nocturnal gastric secretion was studied in twenty-eight patients with active uncomplicated duodenal ulcer. The twelve-hour volume ranged from 550 to 1,750 c.c. with an average of 1,110 cubic centimeters. The free acidity varied from 18 to 122 clinical units, the average being 62. The night secretion was studied in nine of these patients during the period they received x-ray therapy directed at the fundus and body of the stomach. A reduction in volume ranging from 6.3 to 51.6 per cent was obtained in seven patients during the first week of therapy. In two patients an increase in volume was noted. A reduction in free acidity was observed in seven patients. Nocturnal gastric secretion was studied in fifteen patients of the group at varying intervals after therapy. A decrease in volume occurred in fourteen patients, the average reduction being 52.1 per cent. In one patient the volume was increased but the free acidity disappeared. There was a marked reduction in the free acidity of the nocturnal secretion in all patients, the reduction amounting in thirteen of the fifteen to anacidity. A comparable reduction was observed in the gastric secretory response to histamine. These studies indicate that radiation therapy decreases the gastric secretion as measured in the fasting state and by the response to histamine. The duration of the effect is variable.

DISCUSSION

DR. LEON SCHIFF, Cincinnati, Ohio.—I would like to ask how your results in the reduction of volume and acidity of gastric contents compare with those seen following bilateral vagotomy.

DR. MOSES BARRON, Minneapolis, Minn.—I would like to ask if any other method than x-ray was used.

DR. GEORGE WAKERLIN, Chicago, Ill.—Were pepsin studies made on these patients and, if so, what were the results?

DR. LEVIN.—In answer to Dr. Schiff's question regarding the reduction of gastric secretion following x-ray therapy compared to the reduction following vagotomy, of course the effect of x-ray therapy is not permanent. Some patients, I have been told, have had a reduced gastric content for two or three years. It is still too soon to predict what the results of vagotomy will be. I had the opportunity to study one patient who had had vagotomy three years previously. His volume, free acid, and night secretion are still reduced, and there is no free acid in the night secretion.

As to the medical management, all of these patients were on Sippy management. While the studies were being carried on, no patient received any drug that would depress gastric secretion.

Pepsin studies are now being carried out in an effort to determine if that is reduced by x-ray therapy. I am told that there is some reduction in peptic activity.

THE MEASUREMENT OF HUMAN GASTRIC FUNCTION

ARTHUR M. SCHOEN, M.D. (BY INVITATION), AND P. K. KNOEFEL, M.D.,
LOUISVILLE, KY.

Past studies of human gastric function have been concerned largely with the aspiration of gastric contents and the measurement of volume and composition. The actual rate and nature of the secretion cannot be determined from these two observations alone, since gastric emptying occurs. Because of this, others have injected foreign substances into the stomach, such as readily determinable dyes, and have determined more accurately the composition of gastric juice but not the rate of secretion. It is possible to determine the secretory rate and emptying rate if a volume of isotonic NaCl solution (V_o) containing phenol red (W_o) is injected into the empty stomach and the residual contents of the stomach (V_t) containing phenol red (W_t) are aspirated at time (t). Then the volume secreted (S) in time (t) is

$$S = (V_o - V_t) \left\{ \left[\text{Log} \left(\frac{W_t}{W_o} \right) \div \text{Log} \left(\frac{V_t}{V_o} \right) \right] - 1 \right\}$$

and the volume emptied (E) is

$$E = S - V_t + V_o.$$

The necessary assumptions are: (1) No gastric absorption of water or phenol red occurs, (2) no saliva or duodenal contents enter the stomach, (3) there is uniform distribution of phenol red throughout gastric contents during time (t), (4) secretion and emptying occur at constant rates throughout time (t), and (5) gastric contents are completely aspirated at time (t). A satisfactory technique for obtaining gastric juice was developed. This includes preparation of patient for basal conditions, selection and preparation of gastric tube, insertion of tube and determination of location by fluoroscopy, and procedure for complete aspiration. Evidence for the correctness of these assumptions and the validity of the method has been obtained.

From the previously mentioned data and the measured pH and peptic activity of gastric contents, the pH and peptic activity of gastric juice as secreted can be calculated. The resting rate of gastric secretion seen in forty observations in twelve normal persons was 2.09 c.c. per minute, standard deviation 0.96. In one person the daily average basal secretory rate on five days varied from 2.07 to 4.33 c.c. per minute. The average deviation in consecutive observations in the same person was 12.4 per cent. The average hydrogen ion concentration of the secretion was pH 1.51 in twenty-six observations. Studies have been made in patients with peptic ulcer during remissions and exacerbations and in a variety of conditions as aplastic anemia, cholecystitis, pancreatitis, and neurosis. The

responses of normal persons and patients with ulcers to sham feeding, antacids, alcohol, caffeine, histamine, atropine, and insulin have been studied before and after vagotomy.

DISCUSSION

DR. WALTER L. PALMER, Chicago, Ill.—Dr. Schoen and Dr. Knoefel have certainly made gastric analysis scientific. The fallacies of the older methods have been recognized and apparently corrected in this excellent contribution. I wonder if the essayist can answer the question of whether there is a higher level of gastric secretion in a patient with duodenal ulcers than in a normal individual.

DR. SCHOEN.—We have made 161 of these tests. We hesitate to do the work on people who have active duodenal ulcers. We have done some on such patients and those tests have shown definitely that with healing of the ulcer there is a decrease in the rate of gastric secretion.

THE EFFECT OF ENTEROGASTRONE ON FASTING GASTRIC SECRETION AND MOTILITY IN PATIENTS WITH DUODENAL ULCERS

H. MARVIN POLLARD, M.D., MALCOLM BLOCK, M.D. (BY INVITATION), AND W. H. BACHRACH, M.D. (BY INVITATION), ANN ARBOR, MICH.

Enterogastrone has been reported to be effective in the prevention of jejunal ulcers in Mann-Williamson dogs and the prevention of recurrence of peptic ulcer in human beings. The possible mechanisms of its effect are (1) an inhibition of secretion of hydrochloric acid, (2) an inhibition of motility, and (3) an increased resistance of the gastric and duodenal mucosa to ulceration. It has been reported that Mann-Williamson dogs treated for several months with enterogastrone do not exhibit the excessive gastric secretory response to alcohol characteristic of most untreated Mann-Williamson dogs; furthermore, urogastrone has been shown to inhibit the histamine-stimulated gastric secretion in normal human beings. Studies of the effect of enterogastrone on human motility have not been reported.

Our preliminary findings on three patients with intractable duodenal ulcers treated for at least three months with enterogastrone orally show no consistent reduction in the fasting output of hydrochloride by the stomach.

Simultaneous motility and secretion studies after a parenteral dose of 200 mg. of enterogastrone show only a transitory inhibition of motility and secretion. The effect of large doses has not yet been worked out.

The clinical course of the three treated patients has not been followed for a sufficient period of time to evaluate the therapeutic effects of the preparation.

The studies are being continued.

DISCUSSION

DR. JOSEPH B. KIRSNER, Chicago, Ill.—We have been interested in this problem of the effect of enterogastrone on gastric secretion in man. We have not had the oral preparation available. After studying a relatively large number of patients, using larger quantities of the parenteral preparation, we can confirm some of the observations of Dr. Bachrach; that is, the effect of enterogastrone in large doses is rather variable and unpredictable. In certain cases there is a pronounced decrease in the volume and level of gastric acidity even to the

absence of hydrochloric acid in the gastric content. One of the interesting things is that this effect may not become apparent for twenty-four to thirty-six hours after the onset of treatment. In our studies the tube has been kept in place for several days.

Another point I would like to make is that this effect of enterogastrone on gastric secretion is not a fixed one. In one patient with duodenal ulcers, during the course of the experiment an achlorhydria resulted, but during a disturbing psychiatric interview with the patient there was a marked rise in volume of secretion and in the level of hydrochloric acid. Later, after the interview had ended and enterogastrone was continued, there was again a drop in the volume of gastric secretion. This observation illustrates what I am sure is now well known, namely, the effect of the emotions on gastric function.

Dr. Bachrach mentioned an important point with regard to the local pain following the injection of this product. We have found this to be true, and on one occasion, after giving a large dose, the patient continued to be distressed ten days after the injection.

DR. BACHRACH.—We had achlorhydria in two patients, but they both had gastric ulcers. They got well without the benefit of enterogastrone.

EFFECTS OF ALLOXAN UPON THE HUMAN PANCREAS

JEROME W. CONN, M.D., D. L. HINERMAN, M.D. (BY INVITATION), AND R. W. BUXTON, M.D. (BY INVITATION), ANN ARBOR, MICH.

It has now been established that a single dose of alloxan, varying from 50 to 400 mg. per kilogram, produces destructive changes in the pancreatic islets of the dog, monkey, pigeon, duck, guinea pig, cat, and rat. When administered in smaller daily doses a much larger total amount is required to produce evidence of islet cell damage. An occasional animal is found to be refractory.

The only reported observations of the effects of alloxan upon human islet tissue are by Brunschwig and associates (two cases). No histologic changes in the islet tissue were found (1) seven hours after a single injection of 600 mg. per kilogram and (2) one month after a total of 3,150 mg. per kilogram given irregularly over a fifty-day period. The impression has been gained, therefore, that human islet tissue is exceedingly resistant to the damaging effects of alloxan.

Our study was done on a patient harboring a secreting pancreatic insuloma. On each of nine consecutive days she was given alloxan intravenously in the following doses in milligrams per kilogram: 50, 50, 100, 100, 100, 100, 100, 100, 100. Fifteen days after the last injection an islet cell tumor was removed surgically and a section of pancreas was obtained for study.

Microscopic examination of the tissue, stained differentially by several methods, shows no recognizable effect of alloxan upon the islet cells of the neoplasm. However, clear evidence of marked destruction of the islets of the pancreas proper and of early repair are observed. The pathologic changes seen in these islets are typical of those which occur in alloxan-treated animals and resemble most closely those described by Baily and associates as occurring in rabbits given small daily injections for from seven to thirteen days.

The metabolic studies complement the pathologic findings. In the 15-day interval after alloxan and before operation, the insuloma continued to secrete insulin as indicated by the continuation of an extremely low daily level of the fasting blood sugar. In this same period, however, and despite the low fasting blood sugar, a marked decrease in carbohydrate tolerance was apparent. Upon removal of the tumor frank diabetes developed requiring large amounts of

insulin despite low carbohydrate intake. Insulin was discontinued on the forty-fifth postoperative day. To date (sixty-five days postoperative) carbohydrate tolerance has improved but mild diabetes persists.

Conclusions.—

1. In this study the sensitivity of human islet tissue to the destructive effects of alloxan approximates that observed in other species.

2. Neoplastic islet tissue is much more resistant to alloxan than is normal islet tissue.

3. In future attempts to use alloxan therapeutically these facts should be considered.

DISCUSSION

DR. GEORGE GOMORI, Chicago, Ill.—I had a patient dying of carcinoma of the breast with metastases. She was given 500 mg. of alloxan per kilogram of body weight and died thirteen hours later. Autopsy showed no trace of change in the islands of Langerhans. I wonder whether changes shown here cannot be due to massive intravenous infusions of glucose. I have seen a number of specimens from patients who had to be treated with massive doses of glucose. In three or four of these cases the changes were very much like the ones shown here, although the patients did not receive alloxan. Similar degenerative changes are occasionally seen also in animals treated with large doses of insulin. The changes presented here also may be due to massive amounts of insulin released by the tumor.

DR. CONN.—This patient did not receive intravenous glucose therapy in the preoperative period. In attempting to settle the problem of whether hyperinsulinism, per se, may produce similar islet cell changes, we are carrying out long-term experiments in dogs. Preliminary evidence indicates that normal dogs given sufficient protamine insulin daily to keep the fasting blood sugar in the same range as this patient's had been show no islet cell changes similar to those seen in the patient following the course of alloxan.

BOOK REVIEWS

Medical Jurisprudence and Toxicology. Eighth edition. By John Glaister, J.P., D.Sc., M.D., F.R.S. (Ed.), Of the Inner Temple, Barrister-at-Law, etc., Regius Professor of Forensic Medicine, University of Glasgow; formerly Professor of Forensic Medicine, University of Egypt, Cairo; and Medico-Legal Consultant to the Egyptian Government. Williams & Wilkins Company, Baltimore, 1945. Price $8.00. Cloth with 222 illustrations and 691 pages.

This well-illustrated book is intended to cover the main aspects of Forensic Medicine. It is divided into two parts. The first part deals with medical jurisprudence and describes the methods used to determine the cause of death produced by asphyxia, drowning, burning, electricity, hanging, exposure to cold, or by homicidal injuries. Furthermore, injuries produced by firearms, the identification of blood and seminal stains, and the medico-legal aspects of sex crimes are reviewed. The second part of the book is concerned with toxicology. The etiology, the clinical signs, and the treatment, as well as the post-mortem appearances, and the chemical diagnosis of poisonings are described.

The usefulness of the book in this country is limited by the fact that it considers exclusively the British laws. The clinical aspects of toxicology are adequately presented, but many chemical tests described lack specificity or sensitivity and appear to be of relatively little utility in the detection of poisons in biological materials.

ERNEST BUEDING, M.D.

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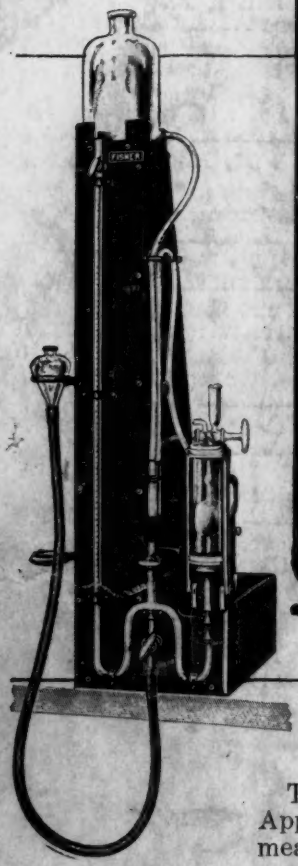

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